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SOME OBSERVATIONS AND EXPERIMENTS ON MALAYAN ANOPHELES WITH SPECIAL REFERENCE TO THE TRANSMISSION OF MALARIA [1]

I, EXPERIMENTAL AND NATURAL INFECTION OF INSECTS WITH MALARIA, WITH SOME NOTES ON THE MORPHOLOGY AND BIOLOGY OF CERTAIN TYPES OF ANOPHELES ROSSI

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(Kuala Lumpur, Federated Malay States)

TWO TEXT FIGURES

The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Board of the Rockefeller Foundation.

The work was done for the most part during 1915 and 1916, and most of the anophelines studied were collected in Selangor State, Federated Malay States. Certain details regarding the method of feeding mosquitoes on gamete carriers, selection of material, dissection, and the like will be given in connection with the tables or included in the description of the technic.

In the identification of Malayan *Anopheles* I have depended chiefly on the publications of Stanton, and I owe much to him for personal aid in identifying certain specimens. The works of Watson, Strickland, Leicester, and other observers in Malaya have also been of much assistance, not only in the classification, but also in the study of biological and epidemiological characteristics of species.

[1] Received for publication September, 1917.

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A good deal of work was done on varieties of *A. rossi*. The large number and the wide distribution of these forms make them of interest from the epidemiological point of view, and the abundance and accessibility of material made them a favorable subject for the study of certain problems in infectivity with malarial parasites.

At the time when these experiments were begun, but one variety of *A. rossi* had been reported from the Malay Peninsula, namely, *A. rossi* var. *indefinitus* Ludl. In the earlier feeding experiments most of the material was collected in brackish water near Port Swettenham. Here the imagoes varied little, and it was assumed, probably on good grounds, that all were var. *indefinitus*. Later a quantity of material was collected from fresh water, in particular from a certain group of fish ponds near Kuala Lumpur. In this lot of material much variability was observed in the breadth of the first black palpal band. There was a continuous series of specimens varying from those exhibiting no such band to those with a terminal black almost equal in length to the terminal white. Some specimens with the broader band together with the corresponding larval skin were submitted to Doctor Stanton, who noted that larva and imago were similar to specimens collected by him in Java. He subsequently sent some of the Java specimens to the British Museum, where they were identified as *A. rossi* Giles, on the basis of the larval characters and of the broader black band. Dr. S. T. Darling had previously noted wide variation in the larvæ of *A. rossi* collected near Kuala Lumpur. It seemed probable, then, that we had to do with two types of *A. rossi*, namely, *A. rossi* var. *indefinitus* and *A. rossi* Giles or a form somewhat similar to it, and further studies were undertaken regarding the characteristics of these two types. For the sake of brevity in description the two Malayan types will be referred to as "type *indefinitus*" and "type Giles."

In the course of comparative infection experiments carried out with these two types and in connection with other observations, over 6,500 larvæ and many hundred adults were examined. Some of the results of these observations are summarized as follows:

Larva.—The chief characteristics of the larva of the two types correspond to the published descriptions of *A. rossi* var. *indefinitus* and of *A. rossi* Giles, respectively. The principal dif-

ference between the two forms in the larva lies in the character and arrangement of the clypeal hairs. In *indefinitus* the anterior internal pair are long, the anterior external relatively short, and the posterior pair very short and situated near the interior pair, not directly behind these but in such a position that lines drawn in a direction directly anterior to the posterior pair would divide the space between the anterior internal into three approximately equal parts. In type Giles the external anterior pair are somewhat longer, and the posterior pair, which are somewhat longer than those of var. *indefinitus*, lie much farther back and are nearly directly behind the anterior internal pair. Some variations in size and position occur in these types, but the combination of characters is such that the observer is rarely left in doubt as to which type a given larva belongs.

While the clypeal hairs of both types are typically simple, I noted various adventitious forkings and branchings, and these were by far more frequent in type Giles. These branchings varied from a simple forking of one of the anterior internal hairs to a branching or forking of four or more hairs and sometimes to a duplication of hairs. The first variation mentioned was by far the most frequent. The branching was noted in mature larvæ, but was more frequent in young larvæ. Some small larvæ with these adventitious branchings were isolated and examined two days later, when they were found to have lost the branches. Larvæ were examined under a cover glass, and where there was any doubt of the presence of these anomalies, specimens were examined under higher powers of the microscope, in order to exclude possible error from the confusion of branching with the appearance afforded by the crossing of hairs.

In 3,019 larvæ of type Giles anomalies of clypeal hairs in some degree were noted in 130, or 4.3 per cent. In 3,525 larvæ of type *indefinitus* they were noted in only 2, less than 0.1 per cent. Possibly many anomalies were overlooked, since the matter was more or less incidental to other observations, but in comparing the two types, both examined in the same way and both of various ages, it is evident that type Giles is more variable in this respect. The matter may be of small weight in itself, but taken in connection with the greater variability of adult type Giles, it goes to show that type Giles is the less stable form. Further we are put on our guard against attaching much specific or varietal value to minor variations in clypeal hairs.

Adult.—The only adult character that appears to be of any value in distinguishing females of the two types is the length of the first black palpal band. The variability of this character is shown in Table I, where females are compared according to the ratio that the length of the first black band bears to the length of the portion of the palp covered by this band and the terminal white band, or

$$\frac{\text{first black}}{\text{first black} + \text{terminal white}}$$

All specimens included in these tables had been previously identified in the larval stage.

In the smaller group under A, of Table I, palp measurements were made under the low power of a compound microscope by means of an eyepiece micrometer. The insects were chloroformed and, while still fresh, placed each on a slide in a definite position; the palps were then covered by a small cover glass in such a way as to bring them parallel to the slide. The same specimens were measured by means of a hand lens without a scale. The hand lens measurements are entered under B, of Table I, for comparison. The line of demarkation between the bands, usually definite under the hand lens, sometimes appeared irregular under the high power. Further, terminal scales that do not get their full value under the lens may be included in the high-power measurement. The two sorts of measurement, therefore, cannot be expected to agree exactly. However, the aim was simply to compare approximately the variability of the two types in regard to a certain character, and in that respect the hand-lens measurement appears to agree closely enough with the more accurate method to suffice. In C larger numbers are included and only hand-lens measurements are given. Since many of the anophelines included under C were to be used subsequently for infection experiments, it is obvious that they could be examined only in the living stage. They were measured in test tubes, usually but one or two in a tube, and were viewed as nearly as possible at right angles to the palps. The hand-lens measurements are only approximate, but as stated above, the approximate agreement of measurements under A and B of the table indicate that the hand-lens measurements were sufficiently accurate to show, in a general way, the amount and character of the variability in length of the terminal palpal bands of the two forms.

TABLE I.—*Palp ratios of different varieties of A. rossi. Incidence of cases.*

A. EYEPIECE MICROMETER MEASUREMENT.

Ratio.	Type Giles, brackish water.	Type Giles, fresh water.	Var. *indefinitus*, fresh water.	Total.	Ratio.	Type Giles, brackish water.	Type Giles, fresh water.	Var. *indefinitus*, fresh water.	Total.
0.00	----------	----------	2	2	0.25	2	4	1	7
0.01	----------	----------	1	1	0.26	2	5	1	8
0.10	----------	----------	2	2	0.27	3	6	----------	9
0.11	----------	----------	2	2	0.28	3	1	1	5
0.12	----------	----------	1	1	0.29	6	4	1	11
0.13	----------	1	----------	1	0.30	10	1	----------	11
0.14	----------	1	2	3	0.31	4	4	----------	8
0.15	----------	----------	3	3	0.32	3	4	----------	7
0.16	----------	1	2	3	0.33	7	4	----------	11
0.17	----------	----------	4	4	0.34	7	----------	----------	7
0.18	----------	1	7	8	0.35	5	----------	----------	5
0.19	----------	1	4	5	0.36	5	----------	----------	5
0.20	----------	----------	6	6	0.37	1	2	----------	3
0.21	----------	----------	5	5	0.38	4	----------	----------	4
0.22	2	2	5	9	0.40	3	1	----------	4
0.23	1	2	1	4	Total	70	49	53	172
0.24	2	4	2	8					

B AND C. HAND-LENS MEASUREMENT.

Ratio.	B. Same specimens as A.				C. All specimens, including A and B.		
	Type Giles, brackish water.	Type Giles, fresh water.	Var. *indefinitus*, fresh water.	Total.	Type Giles, brackish and fresh water.	Var. *indefinitus*, fresh water.	Total.
0.0	----------	----------	2	2	----------	15	15
0.1—	----------	----------	----------	----------	1	12	13
0.1	----------	----------	2	2	2	37	39
0.1+	----------	----------	2	2	7	47	54
0.2—	----------	1	5	6	14	120	134
0.2	----------	2	15	17	64	209	273
0.2+	4	8	21	33	191	61	252
0.3—	23	13	4	40	118	9	127
0.3	17	12	----------	29	109	4	113
0.3+	19	7	----------	26	97	1	98
0.4—	7	6	----------	13	64	----------	64
0.4	----------	----------	----------	----------	48	----------	48
0.4+	----------	----------	----------	----------	25	----------	25
0.5—	----------	----------	----------	----------	3	----------	3
0.5	----------	----------	----------	----------	13	----------	13
Total	70	49	51	170	756	515	1,271

It will be observed in Table I that type *indefinitus* has the narrower range and, in general, the less variability. The amount of overlapping of the two types is such as to indicate the impossibility of differentiating female adults of these types by palpal ratios alone, unless the first black band should have a length near the maximum of type Giles or the minimum of type *indefinitus*. No other adult character has been thus far noted sufficiently constant to differentiate these forms. Type Giles of brackish-water origin seems to have less variability than the fresh-water forms. However, all of the brackish-water type Giles were collected in one locality, and collections from a variety of brackish-water habitats might show a greater amount of variability. To sum up, the data given in this table indicate that it is difficult, if not impossible, to describe adults of certain closely related members of the *rossi* group without recourse to the statistical study of many specimens. Larval characteristics must be taken into account in differentiation, and possibly breeding experiments will be necessary to furnish the final data for the classification of some more closely related types.

Habitat.—The larvæ of type *indefinitus* of Malaya are most commonly found in muddy pools exposed to the sun, and the greater part of my material was collected in such places. *Anopheles rossi* Giles of India is reported to frequent similar places. In Malaya, however, so far as my observation has gone, larvæ of type Giles are never found in such pools, but frequent ponds or large pools relatively clear and supplies with grass or other vegetation. They are commonly found associated with *fuliginosus, barbirostris,* and *sinensis*. In the collection of many hundreds of larvæ in small muddy pools, I have never once found this type. Type *indefinitus,* while commonest in muddy pools, seems to be less selective in its habitat and is found in a variety of places. It is sometimes associated with type Giles in ponds. On a number of occasions I have found small muddy pools containing only type *indefinitus,* while a few feet away ponds contained type Giles in abundance.

The relatively restricted habitat of type Giles, especially its absence from small muddy pools, would be evidence that it is phylogenically distinct from type *indefinitus* and would raise the question as to whether it may not differ, biologically at least, from *A. rossi* Giles of India.

No microscopical character was noted that was of much use in distinguishing the two types of Malaya in collecting. Type *indefinitus* of muddy pools seems slightly larger than type Giles,

but when both are found together in ponds they appear indistinguishable to the naked eye. When occurring in ponds, in the younger stage both much resemble *A fuliginosus* macroscopically.

Type Giles appears to be rather common in the Federated Malay States. It was found by me in seven different places within 6 or 7 kilometers of Kuala Lumpur. In one of these places—certain fish ponds—they could be collected in large numbers throughout a period of several months, and they were abundant in two other ponds. I also found them in fresh water at Tronoh, Perak. In brackish water many were collected in some pools well supplied with algæ at Port Weld, Perak. The brackish-water larvæ seemed a little darker in color and the adult slightly darker than in the fresh-water type, though probably this was only a slight local variation. The occurrence of this type in brackish water, the ordinary breeding place of *A. ludlowi* in Malaya, is noteworthy, since the larva of type Giles and that of *ludlowi* appear identical. It is evident that adult as well as larval stages are necessary in distinguishing these forms.

SUSCEPTIBILITY TO PARASITES OF MALARIA

In comparing the two types of *rossi* in regard to susceptibility to malaria, five pairs of caged anophelines were fed on gamete carriers, one member of a pair type Giles, the other, type *indefinitus*. All specimens were examined in the larval stage and the types separated before emergence. Each pair of cages was fed at the same time and on the same gamete carriers. In two of the pairs both types were collected from the same pond and at the same time. The members of a pair are almost exactly comparable, except that in two pairs some mosquitoes that emerged late were introduced into cages after the cages had been once exposed to a carrier. The majority of those introduced later were of type Giles, so that any error from this source, if such exists, would tend to reduce the percentage infected of type Giles. In every pair but one type Giles showed at dissection much the higher percentage of infected mosquitoes. In the exception 3 out of 4 *indefinitus* dissected were infected and none out of 3 type Giles. The results of this comparison are summarized in a small table appended to the bottom of Table II. It will be seen that of type Giles 37.2 per cent were infected of 94 dissected, while of type *indefinitus* only 11.0 per cent were infected of 73 dissected. Type Giles gave on the average twice

as many zygotes per infected mid-gut and a larger percentage of sporozoites than type *indefinitus*. The only sporozoites found in *indefinitus* occurred in the mid-gut and were apparently degenerate.

A curious difference was observed in the tendency to development of ova in the two types. In 3 out of 5 cages and in 39.5 per cent of the total number dissected type Giles showed ova well advanced in development, often as early as the sixth day after feeding, while well-developed ova never appeared in type *indefinitus*. In 4, at least, of the 5 pairs males were included with the females in cages.

Some further observations on these two types and on other types of *rossi* will be made in connection with certain tables and in the summary at the close of this paper. From the comparative observations on the two types there seems to be substantial evidence for regarding them as distinct forms, biologically and morphologically as well, even though they may not be distinguishable in the adult stage. Further the question may be raised whether type Giles of Malaya may not be a different form from *A. rossi* Giles of India. In view of the great variability of the Malayan type, its restricted breeding places, and its susceptibility to infection with malarial parasites, it may be at least a biological variant, its characteristics dependent in some measure on its topographical environment.

In Table II are summarized the laboratory experiments on infection with malarial parasites of various species of Malayan *Anopheles*. Only controlled lots are included in this table, that is, lots in which at least one insect of some species became infected at a feeding. In other words, one or more of the gamete carriers used had viable gametes at the time the feeding was done. In regard to the species included, *A. ludlowi* is the common brackish-water species of Malaya, having a larva like that of type Giles and an adult resembling *A. rossi*, but with distinctly speckled legs. In the specimens I have observed the length of the first black palpal band is similar to that of the broader banded specimens of type Giles as described above, but apparently *ludlowi* varies less than type Giles in this respect. *Anopheles ludlowi* of Malaya seems to be usually a brackish-water type, though I took some specimens in a large cement-lined reservoir on Kuala Selangor Hill, far above high tide, though near the sea. Whether the Malayan *ludlowi* is identical with the form described by Ludlow from fresh water in the Philippines,

only a study of considerable numbers of the two types will show.

Of the types of *rossi* included in the table, *A. rossi* var. *indefinitus* and *A. rossi* type Giles refer to type *indefinitus* and type Giles compared above. "Known" means that forms were identified by examination of both larva and adult, and "probable" refers to such specimens as were identified by adult characters alone taken in connection with the character of the breeding place. The coast *indefinitus* all came from brackish water, and the character of the adult, of the breeding places, and of such larvæ as were examined makes it practically certain that they were all of the *indefinitus* type and not mixed with type Giles. This is the more likely, since practically all came from one locality where type Giles was not found. The inland "probable" is also almost surely of the *indefinitus* type, since all were collected in the small muddy pools where, as stated above, type Giles was never found. The Giles inland "probable" is less certain, but of a large series of larvæ subsequently collected from the same habitat, practically all were of type Giles on microscopical examination. The mixed group needs no comment. The less certain forms were included, since all were *rossi*.

No other species needs special comment except *hunteri*, a form recently described by Strickland [3] and closely allied to *A. separatus* of Leicester.

The columns in Table II under dissection of salivary glands include practically none dissected before the tenth day after feeding on the gamete carrier. The percentage with infected salivary glands is based only on specimens with infected gut. Practically every specimen with sporozoites in the salivary glands had oöcysts, empty or otherwise, in the mid-gut, and it is believed that the percentage of gut-infected specimens that showed sporozoites in the glands gives a more definite idea of the tendency of a species once infected to form sporozoites than would a percentage based on all dissections, many of them negative. From the percentage of dissections with ova well developed (the last column of the table), dissections of specimens caught in the adult stage are excluded, a large percentage of which already had ova well formed at the time of first feeding. The *rossi* type *indefinitus* and type Giles compared in the small table appended at the bottom are included in the body of the table as well.

[3] *Indian Journ. Med. Research*, 4, 2.

TABLE II.—*Anophelines infected in the laboratory. Controlled series. All dissections.*

Species of *Anopheles*.	Cages.	Mid-gut.				
		Dissected.	Infected.		Number with sporozoites.	Average zygotes per positive mid-gut.
				Per cent.		
A. ludlowi	20	69	42	60.9	2	19.0
A. rossi var. *indefinitus*, coast	28	129	31	24.0	1	12.1
A. rossi var. *indefinitus*, inland "known"	6	85	8	9.4	[a]1	3.3
A. rossi var. *indefinitus*, inland "probable"	3	42	2	4.8	0	2.0
A. rossi type Giles, inland "known"	7	113	37	32.7	4	[b]6.6
A. rossi type Giles, inland "probable"	19	159	45	28.3	16	9.6
A. rossi type Giles, *indefinitus*, inland mixed	9	160	26	16.6	4	3.3
Total all *rossi*	72	668	148	21.5	26	7.9
A. kochi	11	20	9	45.0	0	[c]25.2
A. aconitus	4	6	4	66.7	1	34.5
A. fuliginosus	5	9	3	33.3	1	4.0
A. maculatus	4	10	7	70.0	0	5.3
A. kawari	4	14	10	71.4	1	5.2
A. umbrosus	21	143	25	17.5	[d]5	3.6
A. hunteri	4	8	1	12.5	0	1.0
A. barbirostris	10	107	3	2.8	1	7.0
A. sinensis	6	29	1	3.4	0	3.0
Total	[a]108	1,103	253	22.9	37	10.6
COMPARATIVE SERIES. INCLUDED IN THE ABOVE.						
A. rossi type *indefinitus*	5	73	8	11.0	[b]1	3.3
A. rossi type Giles	5	94	35	37.2	3	6.9
Total		167	43	25.6		6.5

[a] Sporozoites apparently abnormal.
[b] One "very many" counted as 50.
[c] Two "very many" counted as 50 each.
[d] In three cases possibly infected before exposure to gamete carrier.
[e] Subtracting those counted twice.

TABLE II.—*Anophelines infected in the laboratory. Controlled series. All dissections*—Continued.

Species of *Anopheles*.	Salivary glands, dissections 10 days after feeding.				Dissections with ova well developed.[a]
	Dissections.	With sporozoites.	Dissections with mid-gut positive.		
			Number.	Infected salivary glands.	
				Per cent.	*Per cent.*
A. ludlowi	23	3	13	23.1	48.4
A. rossi var. *indefinitus*, coast	87	0	12	0.0	7.8
A. rossi var. *indefinitus*, inland "known"	16	0	1	0.0	0.0
A. rossi var. *indefinitus*, inland "probable"	0	0	0	0.0	4.8
A. rossi type Giles, inland "known"	19	2	11	18.2	30.1
A. rossi type Giles, inland "probable"	125	14	42	33.3	32.4
A. rossi type Giles, *indefinitus*, inland mixed	24	0	18	0.0	11.6
Total all *rossi*	221	16	84	19.0	
A. kochi	0	0	0	0.0	0.0
A. aconitus	1	1	1	100.0	83.3
A. fuliginosus	2	0	1	0.0	0.0
A. maculatus	0	0	0	0.0	0.0
A. kawari	6	2	4	50.0	0.0
A. umbrosus	26	[b]2	6	33.3	81.4
A. hurleri	0	0	0	0.0	87.5
A. barbirostris	8	1	0	0.0	39.3
A. sinensis	6	0	0	0.0	82.8
Total	293	25	109	22.9	30.8
COMPARATIVE SERIES, INCLUDED IN THE ABOVE.					
A. rossi type *indefinitus*	4	0	1	0.0	0.0
A. rossi type Giles	18	2	10	20.0	39.5
Total	22	2	11	18.2	

[a] Exclusive of those caught in adult stages.

[b] In both cases possibly infected before exposure to gamete carrier.

Most of the data found in Table II require little comment and will be summarized in connection with the species summary at the end of this paper. It may be noted, however, that of species that include a fair number of dissections *ludlowi, maculatus,* and *kawari* have relatively high percentages infected. *Anopheles aconitus* is also high, but the numbers are few. The brackish-water *indefinitus* is well above the fresh water, but both are below *rossi* type Giles. *Anopheles barbirostris* and *sinensis* rank low in percentage infected. *Anopheles ludlowi, rossi* type Giles, *aconitus,* and *kawari* show the more-marked tendency to form sporozoites in the salivary glands. *Anopheles umbrosus* may be included under those readily forming sporozoites, although the specimens with sporozoites in the salivary glands had been caught in the imago stage and may have been infected before feeding, since other specimens taken at the same time and from the same locality showed sporozoites, although not exposed to a gamete carrier. *Anopheles indefinitus* showed no sporozoites in the salivary glands, although fair numbers of the brackish-water type were dissected. *Anopheles maculatus* was largely used as a control, and none were dissected till ten days after feeding on the gamete carrier. The number of specimens of different species showing sporozoites in the mid-gut is included in the table, since the proportion of these gives us some indication of the probability that a species may under some conditions form sporozoites in the salivary glands.

Many cages, including many dissections, were done that are not included in Table II, since in these there was no control to show that the gametes were viable at the time of feeding. The relative numbers of controlled and noncontrolled cages and dissections are given in Table III.

TABLE III.—*Comparison of controlled and noncontrolled series.*

	Controlled.		Noncontrolled.	
		Per cent.		*Per cent.*
Cages	108	49.3	111	50.7
Dissections	1,103	53.2	969	46.8

In a large proportion of the experiments the blood of the gamete carrier was examined on the day of feeding and usually at the time of feeding. In a small proportion of cases the carrier was examined on the day preceding or the day following feeding. No carrier was used, in crescent carriers at least, who did not have a sufficient number of gametes to infect, or 1

per hundred leucocytes at least. Nearly all of the carriers, including those who failed to infect mosquitoes, had a much higher percentage of gametes than 1 per cent. In many cases, no doubt, it was more or less a matter of chance that no infections occurred, but that there is a difference in carriers aside from the number of gametes harbored in the blood, and that the same carrier may vary at different times, will be shown in connection with later tables, especially Tables X and XI. In a considerable percentage of the noncontrolled cages the carrier at some previous feeding had infected some of the mosquitoes exposed.

In Table IV are given the percentages of infection of the mid-gut in both controlled and noncontrolled cages. These percentages are of little value in comparison of species where numbers are small, since chance plays a large part in the result, but where numbers are large, the table may be of use in indicating in a general way the probability that mosquitoes of a given species may become infected through biting one or more of a miscellaneous lot of carriers such as might be found in a malarious community.

TABLE IV.—*Controlled and noncontrolled cages by species.*

Species of *Anopheles*.	Dissected.	Positive.
		Per cent.
A. ludlowi	96	43.4
Var. *indefinitus*, brackish water	344	9.0
Var. *indefinitus*, "known"	165	4.8
Var. *indefinitus*, "probable"	73	2.7
Type Giles, "known"	175	21.1
Type Giles, "probable"	306	14.7
Type Giles, *indefinitus* mixed	245	10.2
A. kochi	31	29.0
A. aconitus	22	18.2
A. fuliginosus	33	9.1
A. maculatus	40	17.5
A. kawari	31	32.3
A. umbrosus	240	10.4
A. hunteri	10	10.0
A. barbirostris	197	1.5
A. sinensis	64	1.6
A. aitkeni	1	0.0
Total	2,072	12.2

In Table V dissections are classified according to the type of gamete harbored by the carrier. Controlled series alone are included.

TABLE V.—*Controlled series. Dissections according to gamete carriers.*

Species of *Anopheles*.	Crescents only.		Benign tertian only.		Benign tertian plus crescents.		Benign tertian plus quartan plus crescents.	
	Dissected.	Positive.	Dissected.	Positive.	Dissected.	Positive.	Dissected.	Positive.
		P. cent.		*P. cent.*		*P. cent.*		*P. cent.*
A. ludlowi	61	65.6	8	25.0				
A. indefinitus, brackish water	101	29.7	28	3.6				
A. indefinitus, "known"	73	11.0			12	0.0		
A. indefinitus, "probable"	42	4.8						
Type Giles, "known"	113	32.7						
Type Giles, "probable"	159	28.3						
Type Giles and *indefinitus*, mixed	160	15.6						
A. umbrosus	117	16.2	8	0.0	18	33.3		
A. barbirostris	82	0.0			10	20.0	15	6.7
A. sinensis	21	0.0					8	12.5
A. hunteri	5	20.0			3	0.0		
A. kochi	18	50.0	2	0.0				
A. aconitus	4	75.0			2	50.0		
A. maculatus	5	100.0			5	40.0		
A. kawari	12	83.3			2	0.0		
A. fuliginosus	3	33.3			5	40.0	1	0.0
Total	976	24.1	46	6.5	57	22.8	24	8.3

It will be noted in Table V that the majority of the carriers harbored crescents. Where two sorts of gametes occurred in one or more carriers in the course of a feeding experiment, there was in most cases doubt as to which kind of parasite infected the mosquitoes. However, in the case of *barbirostris* exposed to three carriers having different kinds of parasites, it seems clear that the mosquito found infected was infected with benign tertian. Well-formed sporozoites were present in the oöcysts in the gut. Only six days intervened between exposure to the quartan carrier and dissection, and only four days intervened in the case of the crescent carrier, periods of time too short for the formation of sporozoites. So it is at least highly probable that the effective gamete was from the benign-tertian carrier in which the interval between feeding and dissection was eight days. In the case of the single positive dissection in *sinensis* the evidence is less clear, but it is highly probable that the single positive mosquito found was infected with subtertian. Only two small oöcysts were found in the gut, these apparently not over 6 days old (measurement 22.1 and 30.6 microns in diameter, respectively), and ten days had elapsed since the last exposure to a benign-tertian or to a quartan

carrier. The arrangement of the pigment in the oöcyst resembled that of subtertian, although I have observed much variability in this character in undoubted subtertian oöcysts. The *sinensis* had been exposed to a relatively "potent" carrier, No. 1997, to be described later. It will be observed in this table that infections were obtained with both benign tertian and crescents in *ludlowi* and in var. *indefinitus* from brackish water.

In Table VI all gamete carriers are classified according to their number and the character of gamete harbored. Carriers are arranged under three columns: Those under A were known to harbor viable gametes at one feeding, at least; those under B appeared in connection with positive feedings, but were never the sole carriers under such circumstances—they must be classified as doubtful; those under C were always negative, never appearing in connection with a positive feeding.

TABLE VI.—*Number and character of gamete carriers.*

Type of parasite.	A, known to be positive.	B, doubtful.	C, negative.	Total.
Crescents	19	20	18	57
Benign tertian alone	2	9	7	18
Crescents plus benign tertian	1	0	1	2
Quartan	0	1	3	4
Total	22	30	29	81
Percentage of grand total	27.2	37.0	35.8	

The high percentages under B and C, of Table VI, are noteworthy, since they indicate the large proportion of gamete carriers who apparently harbor nonviable gametes.

In some cages the females were examined singly in test tubes after the first feeding, and those known to have taken blood were separated and given no further exposure to a carrier. In other cages, and possibly the larger number, insects were exposed twice or more to the same or different carriers and the "blooded" ones were not separated. In *rossi*, at least, there was some indication of a greater mortality subsequently among the "blooded" females, which were taken out immediately after feeding. Further, examination of test cages seemed to indicate clearly that few, if any, of the stronger females failed to take blood when given two or three opportunities, especially if the first feeding was done a day or more after emergence. Apparently few, if any, of the weaker ones that failed to take blood under these conditions lived long enough to be dissected. Of the dissections in the controlled series 282 known to have

taken blood and separated after the first feeding gave 27.7 per cent positives. Of those not separated, but as a rule given repeated feedings, 451 dissections gave 26.6 per cent positive, a percentage little below that of those known to have taken blood.

It may be of interest to know what relation, if any, the number of times mosquitoes exposed to gamete carriers bears to the percentage infected and to the average number of oöcysts per positive mid-gut.

Crescent series alone give the best basis for comparison, and such dissections, all from the controlled series, are summarized in Table VII.

TABLE VII.—*Relation of the percentage infected and the average number of oöcysts per mid-gut to the number of times exposed to a crescent carrier.*

Exposed to carrier.	Dissected.	Positive.	Average oöcysts per mid-gut.
		Per cent.	
Once	415	20.5	9.9
Twice	213	27.2	12.4
Three times	208	18.8	21.8
Four times	102	30.4	4.3
Five times	20	45.0	8.9
Six times	18	72.2	3.2
Total	976	24.1	11.3

Twelve species with greatly varying susceptibility to infection are included in this summary, and the probable error is great; however, the numbers are large enough to indicate that there is no very marked positive correlation of number of feedings to percentage infected or average number of oöcysts, except that in the aggregate those exposed four, five, and six times show a higher percentage of infections than those exposed fewer times.

There was little indication in any series of infection experiments of a new infection occurring in a once infected gut through a later feeding of the mosquito on a carrier. There was some variation in the size of oöcysts, but such variation could be found in insects exposed but once. Further we note in Table VII that the average number of oöcysts does not increase with the number of feedings. The most probable case of a superimposed infection was afforded by a lot of *umbrosus* collected in the adult stage. These showed a fair percentage of specimens infected previous to exposure to a gamete carrier. A lot of these were

exposed to a crescent carrier on two successive days and on each of the three following days to a benign-tertian carrier. Nine days after the first exposure they were dissected. The mid-gut of one specimen showed 9 oöcysts not segmenting and apparently about 9 days old. Sporozoites were present in the salivary glands. In the wall of the gut beside one of the immature oöcysts apparently mature sporozoites were found. There was some evidence here of a superimposed infection of the same insect.

Much evidence may be found in these experiments to indicate that the probability of infecting a mosquito depends on factors other than the number of gametes present in the carrier at the time of feeding. In Table VI, under A, we have 22 positive carriers. In fifty-eight feedings with these carriers the percentage of crescents averaged 14.8 per hundred leucocytes. In the always negative carriers, column C, thirty-four feedings gave an average of 23.2 per cent of crescents. However, 4 of these later feedings were done on insects apparently less robust, since reared from larvæ kept for some time in the laboratory. If we omit these four feedings we have an average of 15.1 per cent of crescents for the negative carriers, a percentage still slightly above that of the positive carriers.

This subject is approached in another way in Table VIII. Here dissections of the controlled lots are summarized with respect to the relation that the percentage infected and the average number of oöcysts per infected mid-gut bear to the number of crescents present in the carriers at the times of feeding. Crescent percentages are arranged in groups, and as many dissections as could be brought into these groups are included in the table. Some dissections had to be omitted, because the mosquitoes had been exposed to carriers of such widely varying percentages that they could not be brought into any group. *Anopheles umbrosus, barbirostris,* and *sinensis* had many negatives, and these were nearly all found in two crescent groups; so, for the sake of comparison, a second series of columns is given in which these species are omitted.

It will be noted in Table VIII that the percentage infected does not increase with the increase in percentage of crescents, but comparing groups in which there was a fair number of infected specimens, we note a steady rise in the average number of oöcysts as the gametes increase. The 75.1–100.0 group is scarcely comparable, since there were only two infected mosquitoes in that group.

TABLE VIII.—*Relation of percentage infected and average number of oöcysts per infected mid-gut to percentage of crescents in carrier at time of feeding.*

Crescents per 100 leucocytes.	All species.			*Anopheles umbrosus, barbirostris,* and *sinensis* deducted.		
	Dissected.	Infected.	Average oöcysts.	Dissected.	Infected.	Average oöcysts.
		Per cent.			*Per cent.*	
0.5- 2.0	22	50.2	3.0	17	64.7	3.0
2.1- 10.0	276	22.5	6.4	219	28.3	6.4
10.1- 25.0	298	18.5	15.9	207	27.0	15.9
25.1- 50.0	91	27.5	20.8	90	28.8	20.8
50.1- 75.0	0	0.0	0.0	0	0.0	0.0
75.1 100.0	16	12.5	6.5	16	12.5	6.5
Total	703	22.2	11.9	549	28.4	11.1

A certain carrier, patient 1997, had at one time the remarkably high percentage of 161.5 crescents. Some of the higher percentages of No. 1997 could not be included in Table VIII, since this carrier was at that time associated with carriers of much lower percentages on the same lots of mosquitoes. Some features of this carrier are of especial interest, and the data are given somewhat in detail. He was a Chinese, male, aged 24, and formerly a coolie on a rubber estate. He had been in the Federated Malay States about two years. He had a history of fever without rigor about one and one-half years previously, but had never before been in the hospital. When he came under our observation he had entered the hospital for chancre and did not know that he had malaria. Certain data on this case are included in Table IX.

This case is remarkable for the high percentage of crescents and for the lack of symptoms of malaria in the presence of many parasites, rings as well as crescents. The fever on July 1 is apparently the only symptom observed that can be ascribed to malaria. This carrier was used for many mosquitoes and was very "potent" in infecting them. Data cannot be given for all cages, since this carrier was generally used in connection with other carriers on the same cages. In one cage he was the sole carrier. This cage gave: *Anopheles kawari,* 6 positive of 7 dissected; *maculatus,* 1 positive, 1 dissected; and *fuliginosus,* 1 dissected, negative. There were four exposures to the carrier, although some mosquitoes were introduced into the cage after the first and second exposures. Gametes ranged from 161.4 per cent to 57.7 per cent. The one successful infection of *sinensis,*

if from a crescent carrier (see under Table V), was from this carrier when gametes were 13.6 per cent. Table IX shows the gradual decrease of spleen and crescents under quinine treatment.

TABLE IX.—*Certain data on crescent carrier 1997.*

Date.	Crescents per 100 leucocytes.	Subtertian rings per 100 leucocytes.	Temperature.	Remarks.
June 24	98.7	Present		
June 25	117.1	....do		
June 26	147.6			
June 29			100.8	Spleen enlarged.
June 30	161.4	About 100	100.4	Entered our ward.
July 1	65.8	About 200	103.4	Quinine 30 grains. Spleen to umbilicus.
July 2	89.2	About 85	99.2	Quinine 30 grains and treatment continued on subsequent days.
July 3	57.7	Few if any	100.0	
July 4			normal	
July 5				Spleen, handsbreadth. Given thymol for hookworm.
July 7	23.2			
July 8	18.0			
July 9	13.6			
July 10	9.3			Spleen two and a half fingersbreadth.
July 11	4.8			
July 12	4.4			
July 13	4.1			
July 14	3.2			
July 15	1.1			
July 16	1.4			Spleen one fingersbreadth.
July 17	0.8			
July 18	0.9			
July 20	0.4			Spleen just palpable.
July 21	0.2			
July 22	0.0			
July 23	0.3			
July 24	0.1			Discharged.

The relation of the viability of gametes to their number in the blood of the patient is of interest on purely scientific as well as on epidemiological grounds, and the data of experiments with several crescent carriers will be given in more detail. Tables X and XI contain the data of three carriers on whom a considerable number of cages of mosquitoes were fed. In Sin Tee, Table X, mosquitoes known to have taken blood were not separated, but two or more feedings were done on the same lot. In Plantation No. 1, Table X, "blooded" females only were included in all species, and there was but one exposure to the carrier.

	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*			*Per cent.*		
November 1, 2, 3[a]	20.7	3.1	5.2	10	90.0	39.6				
November 3, 4, 5	4.4	4.9	3.5	6	100.0	24.8	{b½}	33.3	1.0	b
November 5, 6, 7	3.5	2.2	1.1	6	83.3	5.8				b
November 5, 6, 7	3.5	2.2	1.1	b2	0.0	0.0				

December 13	4.1						d29	0.0	0.0	b
December 14	5.9						11	0.0	0.0	b
December 15	2.6						14	0.0	0.0	b 1
December 16	3.5			1	0.0	0.0	33	0.0	0.0	b

[a] Morning feeding—all others evening.

[b] Imagoes caught fully developed. All others were bred from larvæ or pupæ in the laboratory.

[c] One oöcyst in gut.

		Per cent.		Per cent.		Per cent.		Per cent.
December 6								
December 7	7	0.0	[a]1	0.0				
December 8	8	0.0	[a]3	[b]33.3				
December 9	20	0.0						
December 10								
December 11	1	0.0						

It will be noted in Table X that carrier Sin Tee was capable of infecting mosquitoes even after the percentage of crescents had fallen to 1.1, while carrier Plantation No. 1 apparently ran out of viable gametes and failed to infect even when the percentage of crescents was as high as 7.8 and a fair number of mosquitoes of susceptible species were exposed, all known to have taken blood. Plantation No. 1 was probably getting more quinine than Sin Tee, but as numerous experiments have shown, quinine apparently has little effect on gametes further than to increase the rate of their disappearance.

Table XI includes similar data of another crescent carrier, patient 537. Here only one species was exposed to the carrier, *A. rossi* type Giles "probable" (see discussion preceding Table II). In part of the cages only "blooded" females, separated after one feeding, are included. Other cages were exposed twice on succeeding days, and the females known to have taken blood were not segregated. The number of "blooded" mosquitoes surviving to be dissected appears in the last column of the table, data worthy of note but of little value for comparison, since the time intervening between feeding and dissection varied from three to thirty days.

TABLE XI.—*Gamete carrier 537. All A. rossi.*

Date of feeding.	Crescents.	Times exposed.	Known to have taken blood.	In cage after feeding.	Dissected.	Positive.	Average oöcysts per positive gut.
	Per cent.					*Per cent.*	
February 26	29.9	11	All	76	22	18.2	4.3
February 26 and 27	35.2	2			26	38.5	31.5
February 27	35.2	1	All	31	4	0.0	0.0
February 27 and 28	27.7	2			3	33.3	50.0
February 28	27.7	1	All	20	2	0.0	0.0
February 28 and 29	22.8	2			12	50.0	10.0+
March 2	10.8	1	All	27	4	25.0	2.0
March 3	9.6	1	do	7	2	0.0	0.0
March 3 and 4	5.1	2			1	100.0	1.0
March 4 and 5	1.7	2			13	69.2	3.0

It will be noted in Table XI that carrier 537 also retained viable gametes until the percentage of crescents had dropped to 5.1 and, at the second feeding, to 1.7. In both Table X and Table XI it will be noted that there was a tendency to decrease the average number of oöcysts as the percentage of gametes decreased, but there was no corresponding decrease in the percentage of mosquitoes infected. It will be also noted in both tables that the percentage of positives among mosquitoes

known to have taken blood is little greater than that of those exposed two or three times without segregation of those known to have taken blood.

The explanation of the great variability in the infectivity of gamete carriers independently of the percentage of gametes in the blood is not apparent. Dr. S. T. Darling has suggested that it is due to a disparity in numbers of the sexes of gametes. It may be also conceived that in the presence of a sufficient number of both sexes there is in gametes some biological factor, not apparent morphologically, which determines their fertility. Possibly gametes originating in the same oöcysts, or it may be in the same mid-gut, are less mutually fertile than gametes from more widely differing sources. Analogies will be found in other organisms. Further data will be necessary for the explanation of this problem.

In Table X may be noted the failure of *umbrosus* collected in the imago stage to become infected. Further data on the relation of the source of mosquitoes to their susceptibility to experimental infection with malarial parasites are included in Table XII, where the percentages infected and the average number of oöcysts per infected gut are compared with respect to the source of the insects used. Only controlled series and only the four species of which material was taken in the adult stage are included in the table.

TABLE XII.—*Certain species compared as to the susceptibility of material from different sources.*

Species of *Anopheles*.	Mosquitoes collected in imago stage.			Mosquitoes collected as larvæ or pupæ.		
	Dissected.	Positive.	Average oöcysts.	Dissected.	Positive.	Average oöcysts.
		Per cent.			*Per cent.*	
A. umbrosus	100	6.0	4.5	43	44.2	3.3
A. rossi var. *indefinitus*, brackish water	12	8.3	3.0	117	15.6	12.3
A. ludlowi	2	0.0	0.0	67	62.7	19.0
A. kochi	15	26.7	*16.5	5	100.0	*32.2
Total	129	8.5	9.2	232	41.4	14.5

*Entry recorded as "very many." Here reckoned as 50 oöcysts.

It is seen in Table XII that mosquitoes collected in the adult stage show in every species a lower percentage of infection, and in the total and every species but one a lower average number of oöcysts per infected gut than mosquitoes collected as larvæ or

pupæ and subsequently bred out in the laboratory. Mosquitoes collected as adults are apparently less avid of blood than those from the other source, but this cannot wholly explain the difference in infectivity. In some lots insects taken in the imago stage were known to have taken blood, and but a small proportion became infected. A record was kept of some series of insects caught in the imago stage with regard to the proportion taking blood at one exposure. In 5 lots of *umbrosus*, 44 out of 87 once exposed took blood; in *rossi* var. *indefinitus*, brackish water, 15 out of 20; in *kochi*, 7 out of 8. As a routine, material taken as adults was kept one or two days before exposure to a carrier, in order that they might have time to digest the blood already present in the gut. They were then, as a rule, exposed several times; it is probable that the majority of them took blood on repeated exposures. Whatever the explanation, there is evidence that such insects are less susceptible to infection, and they certainly afford less favorable material for infection experiments.

The periods of time intervening between exposure to gamete carriers and dissection are summarized in Table XIII. Only controlled series are included. Since the same cage was sometimes exposed on several succeeding days, the day groups are made large, so as to include as large a proportion as possible of the dissections. In lots exposed to carriers on a series of days one cannot, of course, determine exactly the period intervening between actual infection and dissection.

TABLE XIII.—*Periods intervening between feeding and dissection.*

Days.	Dissected.	Positive.
		Per cent.
Three to six	258	27.9
Seven to nine	134	23.9
Ten to fifteen	251	33.1
Fifteen and one-half to twenty	87	19.5
Twenty-one to twenty-eight and one-half	13	30.8
Total	743	29.5

Specimens were often dissected in the earlier day periods in order to determine the percentage of gut infections. Where there was evidence of infection in a cage, further dissections were often postponed to a time when sporozoites might be found. This explains the relative fewness of dissection in the 7- to 9-day group as compared with the 3- to 6- and 10- to 15-day groups.

In Table XIV dissections at later periods of 101 mosquitoes are compared with respect to different stages of development of the parasites. Only positive specimens are included, and in nearly all cases at least ten days had intervened between feeding and dissection. In cases where cages had been exposed two or more times, the time in days is given as definitely as possible. For example, the entry "11-11.5" means that the mosquitoes were fed on the carrier on the evening of one day and again on the morning of the next and that 11-11.5 days intervened between those dates and dissection. All positive cases of all species dissected ten or more days after feeding are included in this table.

In Table XIV we note that the first appearance of sporozoites in the gut was eight days after feeding. The species was *rossi*, and it had been given one feeding on a crescent carrier. The 8- to 12-day sporozoites in the gut of *ludlowi* was probably benign tertian, and the 4- to 8-day sporozoites in the gut of *barbirostris* probably benign tertian as well (see under Table V). The first appearance of sporozoites in the salivary glands, twelve to twelve and one-half days, was in a group of *rossi* infected by a crescent carrier (No. 537, Table XI). The 12- to 16-day appearance of sporozoites in *kawari* followed feeding with crescent carrier 1997 (Table IX). In the case in which sporozoites were found in the salivary glands twenty-five to twenty-five and one-half days after feeding (twenty-five days after the last feeding, crescent carrier 537), sporozoites were found in the salivary glands of another specimen of the same feeding that was dissected nine days previously. In another lot fed on the same crescent carrier eleven days intervened between the first and the last finding of sporozoites in the salivary glands. We have here some data as to the length of time sporozoites may remain in the salivary glands.

We note in this table the large proportion of dissections, many of them long after the time of infection, in which only degenerate or much retarded oöcysts were found. In practically all such cases the salivary glands were examined and were always found negative. The evidence for abnormality in these oöcysts is based not only on retardation of growth, but also on their appearance. Abnormal vacuolization and watery or coarsely granular protoplasm were sometimes seen, and in some cases the oöcyst not only remained small after a long period, but also gave the impression of an encysted body. In only one or two cases were the oöcysts themselves parasitized by "black spores" or by some similar organism.

TABLE XIV.—*Later stages of development of parasites. Dissections according to days after feeding.*

Species of *Anopheles*.	Interval after feeding.	Oöcysts only.			Sporozoites.		Total dissected.
		Large, apparently unsegmented.	Segmenting or in prezoösporangial stage.	Degenerate or much retarded. No sporozoites.	In gut only.	In salivary glands.	
	Days.						
A. rossi, type Giles, and *A. indefinitus* mixed; mostly type Giles.	8				1		1
	10	1					1
	10-11	1		1	2		4
	11	1		1			2
	11-11.5				2		2
	12			1			1
	12-12.5			1	2	2	5
	12-13			1			1
	13			1			1
	13-13.5			2		1	3
	13.5-14			1	2		3
	14			2			2
	14-14.5				1		1
	15			1			1
	15-15.5			2			2
	16				1		1
	16-16.5			2	1	3	6
	17			1			1
	17-17.5			2		1	3
	18-18.5					1	1
	19-19.5					2	2
	20			1			1
	20-20.5					1	1
	21-21.5			1			1
	22			1			1
	23-23.5			1		2	3
	25-25.5					1	1
	28-28.5			1			1
Type Giles, "known"	12-13				1		1
	13-14			1	2		3
	14-17			2		1	3
	15-16			2		1	3
A. indefinitus, "known"	13-14				1		1
A. rossi, salt water, probably *indefinitus*.	11	1		1			2
	13	2	2	2	1		7
	14			2			2
A. ludlowi	8-12			1			1
	11			1			1
	13	3	1			1	5
	14	1	1			2	4
	15	1					1
A. umbrosus	4-9				2		2
	6-11		1				1
	9-12	1					1
	11-14	3					3
A. kuwari	9-13	2					2
	12-16					2	2
A. aconitus	10-13					1	1
A. barbirostris	4-8				1		1
A. fuliginosus	10-13				1		1
Total		17	5	36	21	22	101

In the varieties of *rossi*, especially, the proportion of these abnormal oöcysts is large, even in cases dissected sixteen or more days after feeding. Even if we do not regard these oöcysts as degenerate, but merely much retarded in growth, the occurrence of so large a proportion of them must affect the species as a carrier of malaria, since the delay in formation of sporozoites would materially decrease the proportion of infected mosquitoes surviving to transmit infection. In all varieties of *rossi*, 34 mosquitoes exhibiting only abnormal oöcysts were found, while cases that showed sporozoites in either gut or salivary glands totaled only 33. The temperature prevailing during these experiments was high and so nearly uniform that differences in the rate of development of malarial parasites could be hardly ascribed to variations in temperature.

MOSQUITOES INFECTED IN NATURE

In Table XV are summarized by species the results of dissections of mosquitoes caught in the adult stage. None of these were subsequently exposed to a carrier except in the case of a lot of 14 *umbrosus*, of which three had sporozoites in the salivary glands. These fourteen were caught in the hospital of a certain rubber estate and later exposed to a carrier. They were dissected five to eight days after feeding, and three had sporozoites in the salivary glands. Since it is highly improbable that sporozoites could be formed in the salivary glands within eight days, it seems fair to reckon these three with the naturally infected lot. Other specimens of *umbrosus* caught at the same time and place and not subsequently exposed to a carrier showed sporozoites in the salivary glands.

It has seemed worth while in the table to classify the dissections of the different species according to the locality in which the mosquitoes were taken. In all cases the mosquitoes were caught in places where gamete carriers might be expected, but the degree of probability of infection varied greatly in the different localities. The probability was the least in "houses near the coast," although two positive *umbrosus* were found there at different times (at Port Swettenham). The "plantation coolie lines" were in all cases highly infected with malaria. In "hospitals" most of the collections were made in the malaria ward of a large hospital, where many cases of malaria were admitted, or in the hospital of a highly infected plantation.

The degree of development of ova is noted in the table, since this gives some indication of the age of the insect and, consequently, of the probability of its having had time to become infected. A large proportion of the insects was found lurking in buildings in the daytime, but some specimens were taken by lamplight. The latter are indicated in the column under remarks. Such insects in this series showed little development of ova, the wings were little frayed, and the indications were that most of these had freshly emerged and were, therefore, less likely to be found infected.

It will be observed in Table XV that sporozoites in the salivary glands were found only in specimens of *A. umbrosus* and *ludlowi*. In some specimens of *A. umbrosus* the sporozoites appeared abnormally thick and short, but in other specimens they were apparently normal. In the positive specimens of *ludlowi*, sporozoites, wholly normal in appearance and staining, were found in large numbers in the salivary glands, and besides, four large pigmented oöcysts appeared in the mid-gut. The single positive specimens of *maculatus* had two pigmented oöcysts apparently about 6 days old. Of the total 667 dissections of the mid-gut, oöcysts were found in 3, or 0.4 per cent; in 508 dissections of the salivary glands 7, or 1.4 per cent, were positive for sporozoites. In 167, chiefly *A. umbrosus*, the mid-gut alone was examined. In a few cases the gut showed no trace of infection, but sporozoites were found in the salivary glands. It is evidently best to examine both gut and salivary glands if one is to ascertain the total number of positives in naturally infected mosquitoes.

It will be noted that all of the specimens of *A. kawari, maculatus*, and *tessellatus* and a large proportion of the specimens of *aconitus, sinensis*, and *fuliginosus* were taken at night. As stated in the introduction to Table XV, such material apparently includes a larger proportion of recently emerged insects than does material collected in houses by day. The one positive specimen caught by lamplight, *maculatus*, had immature oöcysts only.

In the collections in which at least one infected mosquito was found at the same time and place we have the best basis for comparison of the amount of infection in different species. In this series the numbers taken under such circumstances are too small to afford a sound comparison, but the results may be given for what they are worth.

A. ludlowi	Plantation	3	1	3	1						3

A. tessellatus	Plantation coolie lines	2	0	2	0			0
	Cattle shed	1	0	1	0			1
	Total	3	0	3	0	3	0	1
A. hunteri	Houses, seaport town	2	0			2	0	2
	Grand total	667	e3	508	f7	667	g8	197

A. tessellatus	Cattle shed	1	0	1	0			1
	Total	3	0	3	0	3	0	1
A. hunteri	Houses, seaport town	2	0			2	0	2
	Grand total	667	63	508	17	667	a 8	197

TABLE XVI.—*Positive and negative Anopheles in four collections.*

Collection No.	Positive.	Negative.
1	4 *A. umbrosus*	46 *A. umbrosus.*
2	1 *A. umbrosus*	7 *A. umbrosus*, 1 *A. rossi*, 1 *A. ludlowi.*
3	1 *A. maculatus*	2 *A. barbirostris*, 1 *A. sinensis*, 1 *A. tessellatus.*
4	1 *A. ludlowi*	2 *A. ludlowi*, 2 *A. umbrosus*, 5 *A. rossi.*

Of the 8 positives of different species ova were well developed in 5. In nearly every case where a positive was obtained the average percentage of well-developed ova in both positive and negative dissections was high.

SUMMARY BY SPECIES

Anopheles ludlowi.—Much evidence has been adduced by Christophers and others that indicates that *ludlowi* is an important carrier of malaria in certain coast regions. The high percentage of infections with ready formation of sporozoites observed in the experimental series described in this paper, as well as the finding of a naturally infected specimen with sporozoites in the salivary glands, would go to confirm the evidence already obtained regarding the dangerous character of this species.

Anopheles rossi.—The comparatively high percentage of infections observed by me in the brackish water type of var. *indefinitus* would bring this form under suspicion, although sporozoites are apparently not readily formed. Epidemiological evidence in the coast regions of the Federated Malay States is at fault, since this type of *rossi* is there so commonly associated with *ludlowi* and *umbrosus*, both known carriers.

The var. *indefinitus* collected in fresh water shows a low degree of susceptibility to experimental infection and but little tendency to formation of sporozoites. Neither experimental nor epidemiological evidence indicates that this species is an important carrier.

Anopheles rossi type Giles of Malaya shows a comparatively high percentage of infections in laboratory experiments, and sporozoites are readily formed. Further, as shown in the second part of this paper, this type is capable of infecting man under experimental conditions. Epidemiological evidence from other countries, India in particular, indicates that *A. rossi* Giles is rarely, if ever, a transmitter of malaria. But, as stated in the

earlier part of this paper, there is some evidence that type Giles of Malaya may differ, biologically at least, from *A. rossi* Giles of India. Certainly the local type is easily infected experimentally, while the Indian type is reported to be rather refractory. It is difficult to get satisfactory epidemiological evidence in Malaya in regard to type Giles, since it is there commonly associated with *fuliginosus, aconitus,* and other potential carriers. In one or two instances I have found the larva of type Giles in the same part of a lake in which *maculatus* and *kawari* were found. The immediate vicinity of a certain extensive breeding place of type Giles near Kuala Lumpur was not particularly malarious, but the people in the vicinity, chiefly Chinese, were in the habit of protecting themselves by means of bed nets. In another group of houses half a kilometer away and situated near a breeding place of *maculatus* the people protected themselves in a similar way and were comparatively free from malaria. In both cases the population was relatively stable, and possibly the introduction of a susceptible and less well-protected group of people into either place might be followed by an outbreak of malaria. Type Giles showed a marked avidity for blood in feeding experiments, and it is known to frequent dwellings. These characteristics, taken in connection with the experimental evidence, would bring this type under suspicion.

Anopheles umbrosus.—The evidence obtained in these experiments, both in regard to the artificially and naturally infected insects, would confirm Watson's conclusion that *A. umbrosus* is an important carrier in Malaya. The susceptibility of this species under experimental conditions is relatively low, but it may breed in immense numbers, and evidence from laboratory experiments as well as from the condition of adults caught in nature indicates that it is a relatively long-lived species. No exact experiments were made as to its power of flight, but adults were often found in considerable numbers at some distance from breeding places, so that it is probable that *umbrosus* is a strong flier.

Anopheles aconitus.—Stanton and James have recorded natural and artificial infection of this species. There were but few numbers in my experimental series, but the percentage of infections was high, and sporozoites occurred in the salivary glands. This species is often found in houses and readily takes blood. It may be found at considerable distances from its breeding places, and although a small mosquito is apparently capable of long flight. The evidence goes far to incriminate this species.

Anopheles kochi.—No special study was made of this species, and only such specimens as happened to be collected with other species were exposed to gamete carriers. A high percentage of gut infections was obtained, but none were dissected late enough to observe any formation of sporozoites.

Anopheles fuliginosus.—Stanton reports both natural and experimental infection of this species in specimens collected in Malaya. The numbers in my experiments were small, but in the experimental series one third of the specimens dissected was infected. Sporozoites were found in the gut only.

Anopheles maculatus.—In my series this species was largely used as a control of the susceptibility of other species, and I dissected none late enough to obtain sporozoites. The one gut-infected specimen found in nature has been mentioned in connection with Table XV. However, the works of Watson, Stanton, Strickland, and others have established the fact that this species is one of the most important carriers in Malaya.

Anopheles kawari.—The experiments in this series indicate that this species is highly susceptible to infection under experimental conditions. The percentage of gut infections was high, and sporozoites were formed in the salivary glands. None were found infected in nature, but nearly all of the specimens dissected had probably recently emerged. It is difficult to get satisfactory epidemiological evidence, since this species is so commonly associated with *maculatus.*

Anopheles barbirostris and sinensis.—Both are certainly little susceptible to infection experimentally. Only three infected insects were obtained in a large series of *barbirostris,* and only one was obtained in *sinensis.* Stanton has found zygotes in *sinensis* in nature. In view of the facts that these species may be infected with malaria, that they occur in large numbers, and that they readily visit houses and take blood from man, they cannot be wholly acquitted of carrying malaria, but the low percentage of infection and the epidemiological evidence indicate that neither species is an important carrier in Malaya.

Anopheles hunteri.—The number included in my experimental series is too small to show anything further than that this species may be infected.

In regard to the commoner jungle species of Malaya I have obtained no results on *aitkeni* further than to prove that it will take blood when exposed to a carrier. Of those taking blood, the single one that lived long enough to be dissected was negative, but the larvæ had been long kept in the laboratory before they emerged, and *maculatus* controls bred under the same con-

ditions were also negative. *Anopheles tessellatus* was found abundantly on one occasion, both in jungle and in pools more or less exposed to the sun, but there was no opportunity of testing them on a carrier at that time. Of a small lot of larvæ obtained later, only two adult females were obtained, and both failed to take blood.

In summary, laboratory experiments can only prove the susceptibility of a species of mosquito to malaria under more or less artificial conditions and, in a large series, the approximate degree of susceptibility. However, judging from the agreement of laboratory experiments with other evidence in the case of known carriers, it may be concluded that a high percentage of infections experimentally with the formation of sporozoites in the salivary glands furnishes strong presumptive evidence against a given species. The evidence adduced in connection with *A. rossi* (Table XIV) makes it probable that some species of *Anopheles* may be readily infected with malaria parasites, but offer comparatively unfavorable conditions for their development. On the whole, the experiments included in this paper make it doubtful whether any common species of *Anopheles* in Malaya, with the possible exception of two or three jungle forms, is immune to infection and can be wholly acquitted of carrying malaria under certain conditions.

TECHNIC

No attempt is here made to describe the entire technic employed in this work, but it may be worth while to mention a few modifications of the usual technic that have proved especially serviceable.

Where many larvæ have to be examined, I have found a special kind of slide very convenient. Four small pieces of cork or thick cover glass are cemented to an ordinary slide in such a position as to support a cover glass, say 3/4-inch square, at the corners. These cork supports are made of such a height that a larva placed under the cover is held, but not crushed. As larvæ vary somewhat in size, it is well to have two or three sorts of slides at hand. These are best made in duplicate, in order that one may examine one or two larvæ on one slide, while an assistant is placing other larvæ on a second. It is convenient to have a cover glass that projects a little over the slide, so that it is easily caught in the fingers in transferring it to another slide. I have examined many hundreds of larvæ in this way, and I find that one can work very rapidly by this method and, judging from the subsequent development of the larvæ,

with no injury to the insect. The cover glass makes the use of higher powers convenient, and the details of the larvæ are more clearly seen than when examined without a cover.

In many of these experiments I have used as mosquito cages lantern chimneys as described by Darling. For the greater part, however, I have used the common wooden sieves sold for a few cents each in eastern markets. These were used at the suggestion of Dr. H. P. Hacker, and I have developed the idea and have found these sieves a most useful sort of cage. A hole about one inch in diameter is cut in the middle of the wooden side, and a piece of mosquito netting is tied over the open end of the sieve. When it is desired to introduce into the cage mosquitoes as they are bred out, larvæ or pupæ are placed in a wide-mouthed glass jar, over which a piece of mosquito netting or cloth is tied. A hole is cut in the middle of the cloth about the size of the opening in the side of the sieve. The sieve is then tied firmly over the jar in such a position that its lateral opening communicates with the opening in the cloth on top of the jar. If the jar is nearly filled with water, practically all of the mosquitoes on emerging will enter the sieve. One has then only to remove the sieve, place a flap of its netting over the lateral opening, and push the string down to hold it in place. The cage is then ready to be exposed to a gamete carrier, immediately or after the mosquitoes have been kept long enough to become hungry. Or it is easy to take out the mosquitoes by means of test tubes introduced through the lateral opening and examine them singly before placing them in cages for feeding.

In exposing such cages to a gamete carrier, the carrier lies on his back, the thighs or calves are moistened with a bit of wet cotton, and the cages are placed flat under them in close contact with the skin. A blanket is then placed over the lower part of the patient's body. Towels or other cloths may be tied around the cages so as to secure a better contact with the patient's skin. Several cages may be placed under the same carrier at the same time.

In my experiments feeding was usually done in the early forenoon or in the late afternoon. It was not found necessary to wait until nightfall. Mosquitoes usually fed well if exposed a day after emerging. *Anopheles rossi* often bit well within twelve or fifteen hours after the pupal stage. In one lot of 121 females of *A. rossi* over 85 per cent took blood within less than fifteen hours after emerging.

After feeding, the cages may be placed metal side down on

ordinary dinner plates filled with wet sand or merely partly filled with water. The wooden sides of the sieves keep moist, and further moisture can be secured by allowing a flap of the covering to dip into the water or by placing wet pieces of filter paper on the top. The cages in most of these experiments were placed in a meat safe, which was kept in the laboratory and carefully protected from ants.

No food is necessary if the mosquitoes are to be dissected within a few days. In the case of mosquitoes kept for longer periods, I have followed the method of Darling by feeding with a pinch of white sugar placed on moist filter paper in contact with the top of the cage. The proportion of mosquitoes thus fed that became infected with yeasts or bacteria was much smaller than among those fed on fruit juices of any kind.

For dissection the mosquitoes were removed from the cage singly by means of test tubes. The test tubes, each containing a single mosquito, were plugged and placed in a rack on the table convenient to the microscope. Each specimen was chloroformed immediately before dissection. Where positives were found in the first ones examined, the remainder were sometimes returned to the cage and reserved for dissection after the parasites had further matured.

The chloroformed mosquito may be spilled from the test tube directly on a slide and dissected immediately or after a preliminary examination under the low power of a compound microscope. Very fine sewing needles, ground to a blade at the tip and fixed in a stick, make convenient dissecting needles when nothing else is at hand. Very shallow drops of salt solution are most convenient in dissection. In order to prevent the rounding of the drops, I have used slides carefully cleaned in the ordinary way, and I have not found it necessary to use slides prepared with bile. After the gut is drawn out, I have found it convenient to remove the excess of liquid with a bit of filter paper cleanly cut at the margins. This may be manipulated with the needles under the dissecting lens. The drop is thus rendered very shallow, and the malpighian tubes once drawn back remain in position. More liquid may be added, if necessary, after the cover glass has been adjusted. If the last segment of the abdomen is left on the gut, this will not be crushed too flat by the cover glass. If one wishes to flatten the gut further, one has only to press down the cover with a needle.

With an assistant to chloroform the mosquitoes and keep a slide ready prepared for use, one can dissect rapidly. The dissection itself and the subsequent examination of the gut salivary

glands I have done personally in every case. The gut and salivary glands were always examined with a high dry lens and often with the oil immersion as well. Sporozoites were examined fresh and subsequently stained with Giemsa or some similar stain, in order to get confirmatory evidence through the staining reaction. I have made fair preparations of the gut also with the Giemsa stain. The gut is opened and spread on the slide as one would stretch a skin on a board for drying. If the gut is spread in a minimum of fluid and just at the moment of drying, the wall of the gut may be made to lie in a single layer and keep its position on the slide. After thorough drying, one may stain as with a blood film. In a proportion of cases one may get preparations in which the cells of the gut and those of oöcysts give somewhat the Giemsa values such as are obtained in thin blood films.

The sieve cages described above are convenient in collecting adult mosquitoes. Insects caught in test tubes are introduced into the cage through the lateral opening, which is easily kept closed with a flap of the covering. Such cages, covered with a moist cloth and placed in a basket, may be carried by rail or motor car for hours with little apparent harm to the insects. The cages may be placed on plates supplied with water and kept there for a day or so until the insects have digested the blood often present in the gut at the time of collection. They are then ready for dissection.

II. EXPERIMENTAL INFECTION OF MAN WITH MALARIA BY MEANS OF ANOPHELES ROSSI

At the time these experiments were undertaken, there were no infected *rossi* available of which the larvæ had been examined, so that we lack the crucial test as to which type of malaria *rossi* was used for infecting the experimental cases. However, the evidence points very strongly to type Giles. After feeding infected mosquitoes on the experimental cases, the whole lot exposed was always examined singly in test tubes and the ones that had taken blood were immediately dissected. By this means it was known what individual mosquitoes were infected of those that had bitten the patient. The sporozoite-infected specimens that bit these experimental cases all had the broader type of terminal black band.[4]

Further we have seen in part I of this paper that type Giles

[4] Two that bit case 727 had palp ratios of 0.3 and 0.3—, respectively. The one that bit case 408 had 0.3 (see Table I).

is readily infected experimentally with formation of sporozoites in the salivary glands, while type *indefinitus* is little susceptible to experimental infection. Again the pond where the mosquitoes that were used in these experimental cases were obtained was examined repeatedly during some six months subsequent to these experiments, and nearly 99 per cent of the hundreds of *rossi* larvæ collected were of the Giles type. Such evidence, taken by itself, would not be convincing, since the anopheline fauna of a given pond often varies, especially if the water level varies greatly, but the constancy of this type in the breeding place over such a long period may have some value as confirmatory evidence.

Considering all the evidence, it is practically certain that type Giles *rossi* infected the experimental cases, but one cannot wholly exclude the possibility that one or more of the insects was of type *indefinitus*.

Both experimental cases—Chinese coolies—freely volunteered to submit to those experiments, and both had seen enough of malaria to be fully aware of the possible consequences of infection.

No. 727, male, 26 years of age, born in China, had lived in the Federated Malay States a little less than five years. About two years previous to the experiments he had had one attack of fever with rigors daily, the attack lasting about two weeks. He had no history of fever since. The spleen was normal. He had had secondary eruptions of syphilis about one year previously. As shown in Table XVII, a daily blood examination showed no parasites until the fourteenth day after exposure to infected mosquitoes.

On March 15 and 16 three attempts were made to infect No. 727, but only five mosquitoes took blood. One of these had oöcysts in the mid-gut, but none had sporozoites in gut or salivary glands. On March 17 mosquitoes that had been deprived of sugar for two days were applied and five out of eleven exposed took blood. Two of the five had sporozoites in the salivary glands. The others were negative, in both gut and salivary glands. Dissections were made immediately after feeding. These mosquitoes had been infected by crescent carrier 537 (Table XI) nineteen to nineteen and one-half days previously. Mosquitoes had been exposed twice to the crescent carrier, and on the first exposure, the evening of February 26, the percentage of crescents was 35.6.

Both of the sporozoite-bearing mosquitoes had well-developed ova, a further indication that they were of type Giles, since, in the comparative infection experiments (see discussion preceding

Table II), type Giles showed a fair percentage of cases with well-developed ova at dissection, while type *indefinitus* showed practically none, comparing insects of the same age at dissection.

Experimental case 408, male, 33 years of age and formerly a mining coolie, was born in China and had been in the Federated Malay States four years. He had no history of fever. He had had beriberi three years previously and still had the beriberi gait, but was well nourished and otherwise in good physical condition. The spleen was not enlarged. No parasites were found in the blood for seventeen or more successive daily examinations. On March 20 the mosquitoes remaining from the lot used in case 727 were exposed to No. 408, but none bit, although they had not been given food for five days. On the same date seven of another lot were tried, and none bit. On March 21 the four of the seven remaining alive were tried and two bit. The first bit feebly and took only a small quantity of blood. The second was observed to bite twice, at least, and took a fair amount of blood. On dissection the first showed no parasites, but the second had one empty oöcyst in the gut and sporozoites in the salivary glands. Of another lot of three tried on the same day one bit. It had oöcysts, apparently degenerate, in the mid-gut, but no sporozoites in the salivary glands. So it seems clear that the subject was bitten by only one sporozoite-infected mosquito and that this one bit at least twice. This mosquito was also infected from crescent carrier 537 (Table XI) sixteen to sixteen and one-half days previously. The lot of mosquitoes were exposed twice to this carrier on succeeding days. At the first exposure the percentage of crescents was 5.1, at the second, 1.7.

Various observations on these two experimental cases are compared in Table XVII.

No. 727 showed no rise of temperature until some eight hours after parasites were found in the blood, while no parasites were found in No. 408 until the third day after the rise of temperature. No. 727 showed marked symptoms, headache, severe vomiting, and on the third and fourth days after illness began, severe attacks lasting about twenty minutes characterized by convulsive symptoms, clutching of the fingers, pain in the throat, and rapid and shallow respiration. The spleen was not enlarged at any time in this case. No. 408 had practically no symptoms at all, except that he complained of headache on one day. There was marked enlargement of the spleen. Both cases made a rapid and complete recovery under quinine treatment. Temperature charts of both cases are given.

TABLE XVII.—*Cases experimentally infected with malaria by A. rossi, compared by days.*

CASE 727.[a]

Days after infection.	Parasites.	Maximum temperature.	Symptoms.	Spleen.	Quinine.
		°F.			*Grains.*
1-13	Negative (daily examination).	Normal	None	Normal	Nil.
14	Few at 11 a. m.[b]	100.8[c]	Slight	do	Nil.
15	Increased	101.8	Marked	do	Nil.
16	Little change	103.6	do	Dullness increased	Nil.
17	Marked increased	103.2	Severe	Not enlarged	40
18	Only 1 found	99.2	do	Normal	45
19	1 (?)	Normal	Slight		40
20	Negative		Little or none		30
21	do				
22	do				
23					

CASE 408.[d]

1-13	Negative (daily examination).	Normal	None	Normal	Nil.
14	Negative	do	do	do	Nil.
15	do	do	do	do	Nil.
16	do	do	do	do	Nil.
17	do	100.2	do	Not palpable	Nil.
18	do	101.8	do	Palpable	Nil.
19	Few	101.6	do	Two and a half fingersbreadth.	Nil.
20	Increased	103.2	do	do	Nil.
21	Further increased	99	Slight	Umbilicus	30
22	Negative	Normal	Nil	do	40
23	do	do	do		30

[a] Case 727. Blood examined daily eighteen days after disappearance of parasites, negative. Quinine treatment was continued at least ten days.

[b] For numbers, see Table XVIII.

[c] Began to rise from 4 to 8 o'clock in the afternoon; 100.8° was the midnight temperature.

[d] Case 408. Blood examined daily twelve days after disappearance of parasites, negative. Spleen April 15 (=twenty-fifth day) slightly reduced; April 20, 3 fingersbreadth; April 25, 1.5 fingersbreadth; April 27, palpable below ribs; April 29, palpable, deep respiration only; May 2, not palpable. Quinine treatment was continued eighteen days.

Some details as to the number of parasites found on different days are given in Tables XVIII and XIX. In case 727 an attempt was made to estimate roughly the number of parasites appearing in the peripheral blood on different days. A large number of erythrocytes were counted, or rather their number estimated, in a thin preparation stained by Hasting's stain. The estimate was made by means of a disk inserted into the eyepiece

and so ruled as to divide the field into quarters and eighths. By counting fractional parts of the erythrocytes found in a field and comparing similar fields, an estimate, more or less precise,

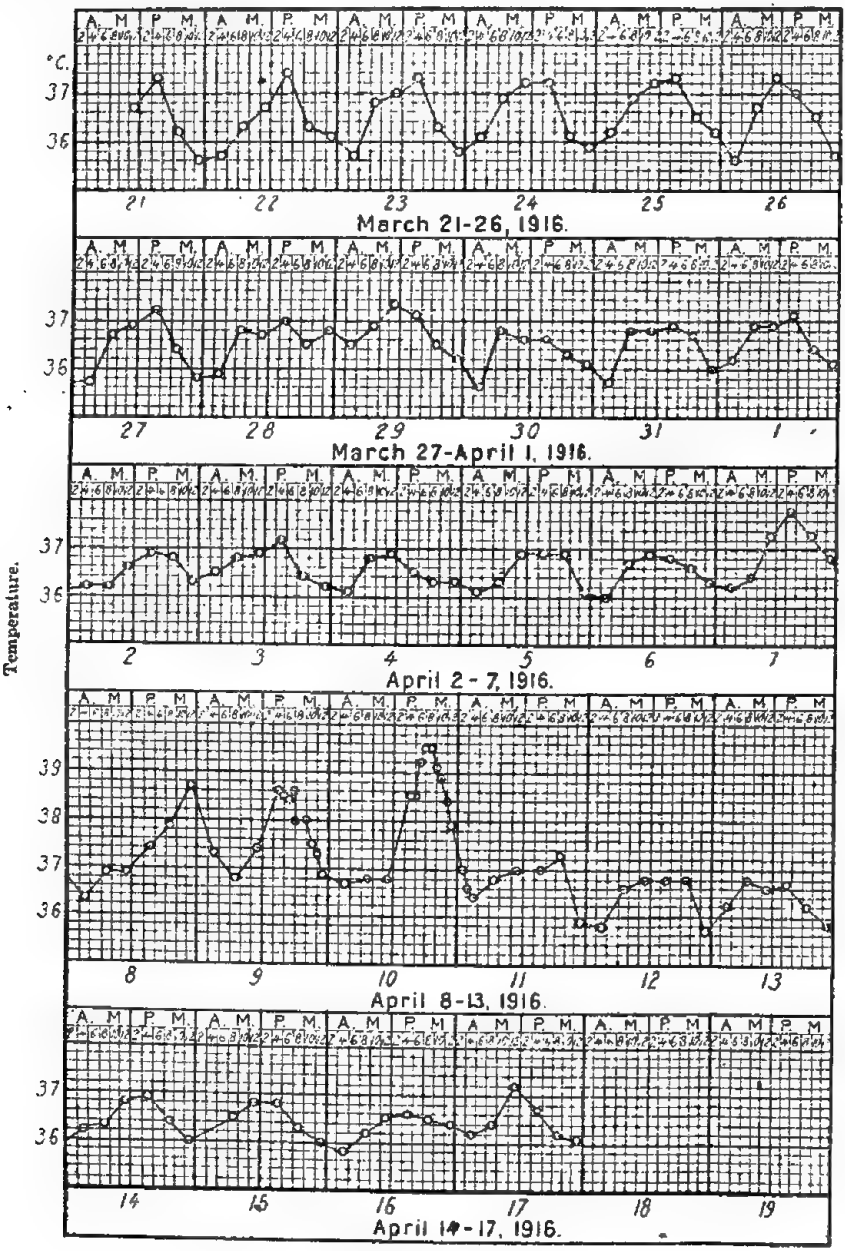

FIG. 1. Temperature chart of Thi Mue.

was made of the proportion of parasitized erythrocytes. For comparison, the number of parasites found in thick films was estimated in terms of the number per 100 leucocytes. The ratio

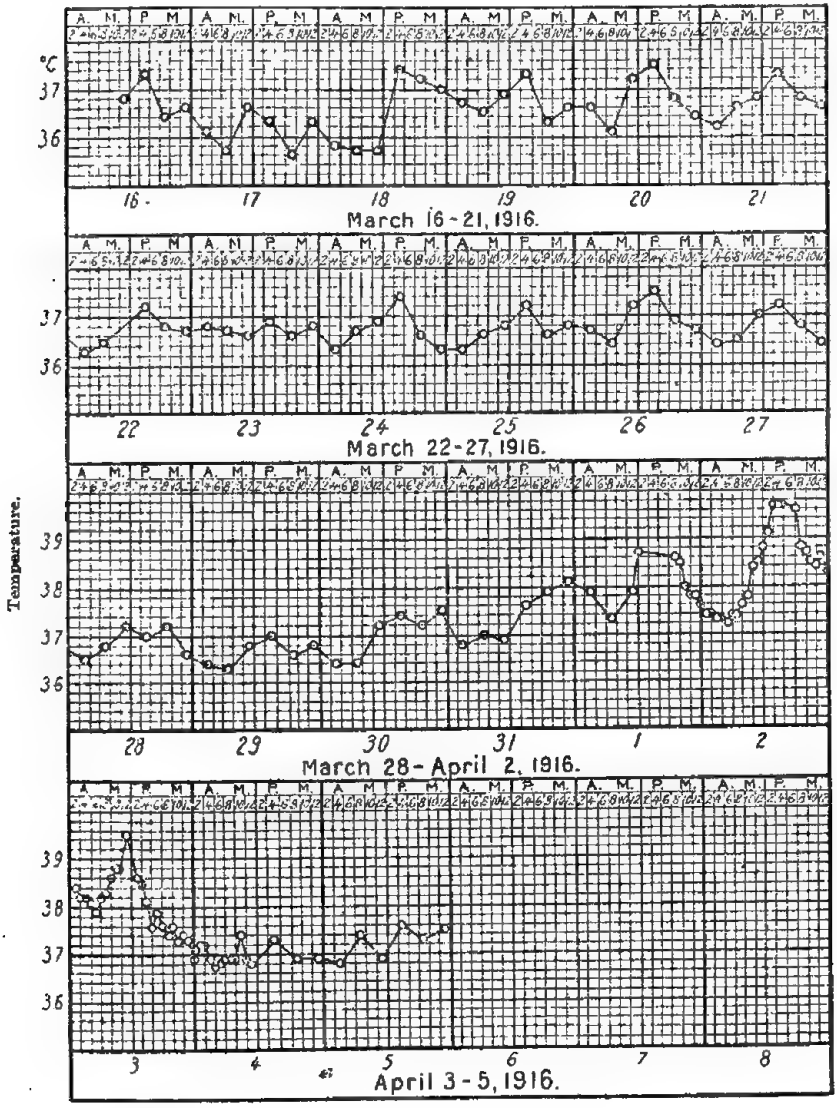

FIG. 2. Temperature chart of Thang Hong.

of daily increase or decrease of parasites as obtained by the two methods is compared. In experimental case 408 only the proportion of parasites in the thick film was estimated.

TABLE XVIII.—*Case 727. Parasites in peripheral blood.*

Date.	Thin film.				Thick film.				Temperature at time of taking blood samples.
	Erythrocytes estimated.	Parasites found.	Parasites per 10,000 erythrocytes.	Ratio of increase or decrease.	Leucocytes counted.	Parasites found.	Parasites per 100 leucocytes.	Ratio of increase or decrease.	
									°F.
March 30		0				0			
March 31, 11 a. m. (14th day)	64,300	3	0.5	1.0	1,000	39	3.9	1.0	98.2
April 1, 10.45 a. m.	47,900	37	7.7	15.4	600	237	39.5	10.1	100.2
April 2, 11.30 a. m.	57,100	34	5.9	11.8	600	337	56.2	14.4	101.4
April 3, noon	18,200	39	21.4	42.8	400	756	189.0	48.9	[a]103.2
April 4		+			500	136	27.2	7.0	Normal
April 5, 11.30 a. m.		0							

[a] Quinine was administered at 4 o'clock in the afternoon.

TABLE XIX.—*Case 408. Parasites in peripheral blood.*

Date.	Thick film.				Temperature at time of taking blood samples.
	Leucocytes counted.	Parasites found.	Parasites per 100 leucocytes.	Ratio of increase or decrease.	
					°F.
April 9, 7.30 a. m. (19th day)	(a)	5			98.6
April 10, 7.30 a. m.	3,000	5	0.17	1	98.6
April 10, 7.15 a. m.	1,000	5	0.50	3	[b]98.2
April 12		0			98.0

[a] Long search. [b] Quinine was administered at 4 o'clock in the afternoon.

It will be observed in Table XVIII that, while there is some correspondence between the ratios of increase and decrease observed in No. 727 by the different methods, the results are at variance, especially on the third day. It seems probable that there was, at least, a diminution in the rate of increase of the parasites on that day. Probably the results given by the thick films are more reliable. The probable error is great in basing results on these small samples, whatever the method used. The results may give us some notion of the rate of increase or decrease of parasites, but only an approximation as to their absolute numbers in the peripheral blood.

In both cases the parasites examined carefully in thin films were evidently subtertian. No crescents were observed in either experimental case.

In summary, the evidence seems clear that these experimental cases were infected with malaria as the result of exposure to *A. rossi* infected in the laboratory. The possibility of a relapse from a former infection must be always taken into account in such experiments when performed in a malarious country. But that such relapses should follow exposure to infected mosquitoes in two cases, one occurring fourteen and the other seventeen days after exposure, would be a remarkable coincidence indeed, especially in view of the fact that both patients had been known to be free from fever many days before the experiments and that both showed the same type of parasite as that that infected the mosquitoes.

It also seems clear that a single mosquito of *A. rossi* may infect at one exposure. The fact that the case that received sporozoites from two infected mosquitoes, and, presumably, the larger dose, showed an earlier appearance of parasites and the more marked symptoms may be only a coincidence, but it is worthy of note. One can do little more than guess at the number of sporozoites injected by a single mosquito, but judging from the number of sporozoites found at dissection after feeding on the experimental cases and comparing with the numbers observed in the salivary glands of many infected mosquitoes of the same species, one would say that the effective number is a matter of hundreds rather than of thousands, and more probably a matter of scores.

It is noteworthy that the case (No. 408) that had the longer incubation period (seventeen days), the fewer parasites, and the less severe symptoms had a marked enlargement of the spleen. No. 727, with the shorter incubation period (fourteen days), the greater number of parasites, and marked symptoms, showed no enlargement of the spleen. This case had a history of a previous attack, while No. 408 had none. It is interesting to compare gamete carrier No. 1997 (Table IX), who showed enormous numbers of subtertian parasites, practically no symptoms, and a marked enlargement of the spleen. He had a history of a previous attack. This small group of three cases certainly serves well to show the great variety of manifestations observable in subtertian malaria.

ACKNOWLEDGMENTS

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ILLUSTRATIONS

TEXT FIGURES

DOES THE IRRITANT ACTION OF EMETINE HYDROCHLORIDE EXTEND TO THE KIDNEY?[1]

By D. DE LA PAZ and R. MONTENEGRO

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Emetine possesses a powerful local irritant action, but its irritant effect on the kidneys and other remote organs, except the gastrointestinal tract, is not definitely known. Duckworth,(2) in 1869, observed the frequent occurrence of albuminuria in animals poisoned with emetia.[2] Zeff(8) found small amounts of albumin in the urine of most of his patients treated with emetine for pulmonary disease. According to Foulkrod,(3) the kidney undergoes some damage, and albuminuria results in animals given emetine. Shortly after the discovery by Vedder(6) and Rogers(5) that emetine is amœbicidal, it has been extensively used in amœbic dysentery in large doses injected repeatedly for a considerable length of time. It is surprising that this method of employing the drug has not apparently given rise to renal complications. Does the irritant action of emetine extend to the kidneys when it is administered as it is usually done in amœbic dysentery? The difficulty of obtaining a satisfactory information from the patients is self-evident. We have, therefore, carried out the present experiments on animals.

Active full-grown dogs were used for the experiments. They were given soap and water baths and rubbed dry with towels, and each was placed in a clean metabolism cage. Their diet consisted of rice, meat, and fish. Food and water were given only once in twenty-four hours at about the same time each day, but the animals were allowed each time as much as they could eat and drink. The urine for twenty-four hours was collected daily in a graduated cylinder and examined for casts and albumin. We used the test for albumin, described by Glaesgen.(4) This offers two advantages over Heller's nitric acid test; it detects the presence of a smaller pathological amount of albumin and gives a sharper reaction. When at least five successive examinations showed the absence of albumin and casts

[1] Received for publication August 11, 1917.

[2] Emetia is the alkaloid of ipecacuanha, according to Duckworth. It is probably impure emetine.

in the urine, four dogs were given hypodermic injections of a 1 per cent solution of emetine hydrochloride crystals (Paul-Merch). Baermann and Heinemann(1) consider that about 4 milligrams per kilogram of body weight are the maximal intravenous dose for man. We gave our dogs 1 milligram per kilogram of body weight. This dose corresponds to 65 milligrams or 1 grain for an adult weighing 65 kilograms; it is, according to Vedder(7) and others, usually sufficient to destroy the amœbæ in the intestine of a dysenteric patient. The injection was repeated once daily until the animals died.

Emetine caused vomiting, a rise of temperature, and hemorrhagic diarrhœa. One dog survived six injections; two dogs, seven; and one dog, eleven. Post-mortem examination revealed congestion of the internal organs, skin, and subcutaneous tissue; hemorrhages in the intestine; and in two dogs, ecchymoses at the sites of injection. Table I gives the results of daily examinations of the urine of four emetinized dogs.

The examination of the urine, as shown in the table, does not give conclusive evidence that emetine has induced inflammation of the kidneys. The appearance of albumin in the urine of dog 1 on the second day of emetine injection and in the urine of dog 3 on the fifth injection cannot be due to inflammatory changes; otherwise it should have appeared on the succeeding days and its quantity should have been increased by the subsequent injections of emetine. We cannot explain the significance of the short hyaline casts that appeared in the urine of dogs 1, 2, and 3. We also observed them in the urine of three saline controls. However, the fact that their appearance was not accompanied by albumin except in one instance (that is, on the fifth injection in dog 3) diminishes their importance as a sign of inflammatory lesion of the kidneys. This is confirmed by the examination of the sections of the kidneys. When examined histologically, the kidneys of dog 1 showed very slight acute parenchymatous degeneration; the kidneys of dog 2 showed congestion and its results, hemorrhages between the layers of the capsules, and slight if any degeneration of the tubular epithelium; the kidneys of dog 3, congestion and slight degeneration of the tubular epithelium only; and the kidneys of dog 4, very little recognizable pathological change on account of extensive post-mortem alteration. The absence of inflammation that emetine readily induces in the eye, respiratory passages, alimentary canal, and subcutaneous tissue indicates that it is not eliminated by the kidneys, or if it is, that it passes through them in very high dilution.

TABLE I.—*Examination of the urines of four emetinized dogs.*

Day of injection.	Dog No.	Urine in 24 hours.	Albumin.	Casts on 1 slide.	Remarks.
		c. cm.			
First	1	200			The amount of urine is approximate. The urine was voided outside of the cage.
	2	390	Negative	None	The amount of urine is approximate. About 150 cubic centimeters were voided outside of the cage.
	3	245	do	do	
	4	250	do	do	
Second	1	200	Trace	do	The amount of urine is approximate. About 30 cubic centimeters were voided outside of the cage.
	2	175	Negative	do	
	3	325	do	do	
	4	130	do	do	
Third	1	165	do	do	
	2	480	do	do	
	3	160	do	do	
	4	65	do	do	
Fourth	1	0			
	2	372	Negative	None	
	3	100	do	4 short hyaline	
	4	80	do	None	
Fifth	1	290	Abundant	do	Albumin probably came from the bloody stools found in the cage.
	2	160	Negative	do	
	3	170	Trace	Several short hyaline	
	4	80	do	None	Albumin probably came from the bloody stools found in the cage.
Sixth	1	90	Negative	do	
	2	125	do	do	
	3	150	do	2 short hyaline	
	4	50	do	None	The last injection was given this day. The dog was found dead on the next day.
Seventh	1	65	do	1 short hyaline	Do.
	2	230	do	None	
	4	180			Urine was mixed with bloody stools. The dog was found dead.
Eighth	2	63	Negative	1 short hyaline	
Ninth	2	125	do	Several short hyaline	
Tenth	2	120	do	do	
Eleventh	2	85			Urine was mixed with bloody stools. The dog was found dead on the next day.

Six dogs were used as controls; two received hypodermic injection of 6.7 milligrams of uranium nitrate per kilogram of body weight for two days in succession, and the remaining four received daily injections of sterile saline solution. The saline controls were autopsied after they had received the same number of injections as the emetinized animals. Saline solution did not produce hemorrhages at the places of injection nor abnormal changes in the urine and the kidneys, except the appearance of the short hyaline casts that were noted in the urine of three dogs already referred to. Their appearance cannot be ascribed to the injection of saline solution, because they were observed to appear periodically in the urine of these dogs even before the injections. Uranium nitrate caused the appearance of albumin, desquamated renal cells, granular casts, and finally anuria. When autopsied seven days after the first injection of uranium nitrate, one dog showed hemorrhage at the point of injection, anasarca, and acute tubular nephritis, while the other showed acute diffuse nephritis involving especially the tubules.

CONCLUSIONS

Our results show that emetine hydrochloride gave rise to congestion and slight parenchymatous degeneration of the kidneys. While in one dog the drug produced hemorrhages at the sites of injection and between the layers of the renal capsule, and at the site of injection in another dog, in no case did its irritant action extend to the parenchyma of the kidneys, although we administered it in a quantity that, when injected daily, eventually caused the death of the animals.

We wish to thank Professor B. C. Crowell, of the department of pathology and bacteriology, University of the Philippines, for examining the sections of the kidneys.

REFERENCES

(1) BAERMANN, G., and HEINEMANN, H. *Münch. Med. Wochenschr.* (1913), 60, 1210.
(2) DUCKWORTH, DYCE. *Rep. St. Bartholomew's Hosp.* (1869), 5, 244.
(3) FOULKROD, cited by PELLINI, E. J., and WALLACE, G. B. *Am. Journ. Med. Sci.* (1916), 152, 325.
(4) GLAESGEN. *Münch. Med. Wochenschr.* (1911), 58, 1123.
(5) ROGERS, LEONARD. *Brit. Med. Journ.* (1912), 1, 1424.
(6) VEDDER, E. B. *Bull. Manila Med. Soc.* (1911), 3, 48.
(7) IDEM. *Journ. Am. Med. Assoc.* (1914), 62, 501.
(8) ZEFF, cited by PELLINI, E. J., and WALLACE, G. B., op. cit.

REVIEWS

The Diagnostics | and | Treatment of Tropical | Diseases | by | E. R. Stitt, A. B., Ph. G., M. D. | [10 lines] | second editon | revised and enlarged | with 117 illustrations | Philadelphia | P. Blakiston's Son & Co. | 1012 Walnut street | Copyright, 1917. Pp. i–xiii+1–534. Cloth, $2 net.

The second edition of Stitt's Diagnostics and Treatment of Tropical Diseases has been brought well up-to-date by the addition of chapters on typhus fever and Rocky Mountain spotted fever in Part I, of one chapter dealing with special diagnostic problems in the tropics, and of another discussing the diagnostic value of clinical manifestations of diseases in the skin and organs of special sense. The work as a whole is to be commended as a clear and concise description of diseases incident to the tropics.

J. A. J.

Diseases | of the | Stomach, Intestines, | and | Pancreas | by | Robert Coleman Kemp, M. D. | [9 lines] | third edition, revised, with 438 illustrations | Philadelphia and London | W. B. Saunders Company | 1917 | Pp. 1–1096. Cloth, $7 net; half morocco, $8.50 net.

From the preface to the third edition: In view of the great value of the *x*-ray as an aid to diagnosis in the gastrointestinal tract, a special section has been devoted in this new edition to the radiography of gastric ulcer, gastric cancer, duodenal ulcer and gall-bladder disease and in addition, there are a large number of radiographs of other conditions.

Many physicians have neither time nor opportunity to devote to a practical clinical course, and next in value to this for the purpose of instruction is the employment of photography to demonstrate the methods of diagnosis and treatment. Of this, advantage has been taken.

As visceral displacements have recently assumed an important position, their symptoms, diagnosis, and treatment, notably by mechanical methods, are specially described. Typhoid fever is included in this volume on account of its intestinal complications and for the purpose of differential diagnosis.

A chapter is devoted to Diverticulitis, which has become an important subject.

The endeavor has been made to indicate as clearly as possible the conditions that call for surgical procedure.

Asthma | presenting an exposition | of | the nonpassive expiration theory | by Orville Harry Brown, A. B., M. D., Ph. D. | formerly assistant professor of medicine, St. Louis University | with a foreword by | George Dock, Sc. D., M. D. | professor of medicine, Washington University Medical School, St. Louis | Thirty-six engravings | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1–330.

The author presents in this volume an extremely comprehensive study of "Asthma." His theory of the condition, the "Nonpassive Expiration Theory," is well conceived and is clearly stated and certainly should excite further study and research. The book is based on nine years' study of asthma, and the theory, for which the author claims "that preasthmatic states can be recognized thereby and successfully combated," certainly deserves careful consideration by practitioners. Not the least pleasing feature of the work is the author's style. The work is heartily commended to the student and especially to the general practitioner, who as a rule needs something of this character to rouse him.

Doctor Brown does not play up his own theory to the exclusion of others, but gives a historical view of the subject and discusses very numerous ancient and modern theories of asthma as well as the many remedies and treatments. The latter seem to be about as numerous and varied as the theories. Almost everything from electric-light baths to diphtheria antitoxin and from resection of the fifth and sixth ribs to doses of pulverized human skull in water seems to have been used in attempts to relieve asthmatics.

The bibliographies at the ends of chapters are very full. For example, there are 470 citations following the chapter on historical observations and theories, 213 following the chapter on treatment, and 70 following clinical history, physical signs, and symptoms. The general index is a useful addition. The index to authors contains 724 names and many more entries.

The book is illustrated with a variety of engravings. Some are reproduced from kymograph records and from drawings; others are half tones from Roentgenograms. The book shows care and good taste in the printing, and the text paper is without gloss.

J. A. J. and R. C. McG.

Traumatic Surgery | by | John J. Moorhead, B. S., M. D., F. A. C. S. | [4 lines] | with 522 original | illustrations | Philadelphia and London | W. B. Saunders Company | 1917 | Pp. 1–760. Cloth $6.50 net; half morocco, $8 net.

From the preface: This book is written with the main idea

of placing in one volume the information necessary to diagnose and treat all the usual and most of the unusual effects of accident and injury.

The profession at large has become reawakened to the problems of accident surgery, and, incidentally, has come into a new relationship with the injured because of the operation of compensation and allied laws; likewise, the victims of accident, and civic, judicial, legal, and other agencies are exacting from the physician a higher grade of care and placing on him an added burden of responsibility.

The text also aims to state the measures which the writer has found most practical in his own experience, and an effort has been made to unify and standardize the treatment of such common injuries as wounds, infections, burns, and the usual fractures. It will be noted that stress is placed on the routine use of but few antiseptics, the drainage of all wounds, the immediate and complete reduction of fractures, and non-reliance upon complicated splints or those that hide the part or are irremovable.

The writer believes that open air and sunshine is the best treatment for any infected wound in any location from any source, because purulent secretion is soon checked, there are no pus-soaked or wound adhering dressings (literally pus poultices), and the comfort of the patient is measurably increased and healthy granulations and minimum scarring occur promptly. For many years now this plan has been employed, and the writer is convinced that its efficacy is best proved by the statement that skin-grafting has not been necessary since this form of aërotherapy and heliotherapy has become routine in his practice.

Handbook of Gynecology | for Students and Practitioners | by | Henry Foster Lewis, A. B., M. D. | [5 lines] | and | Alfred de Roulet, B. Sc., M. S., M. D. | [3 lines] | With one hundred and seventy-seven | illustrations | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1–452.

Experimental | Pharmacology | by Dennis E. Jackson, | Ph. D., M. D. | associate professor of pharmacology, Washington University | Medical School, St. Louis | With three hundred ninety original illustra- | tions including twenty-four full-page | color plates | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1–536.

Darwin was so repulsed by the study of geology because of the poor presentation of the subject that he resolved never again to have anything to do with it. Fortunately he revised his decision. One of the reviewer's pet theories is that the first presentation of a subject is the most important for the student. If this theory is sound, the laboratory manual holds a responsi-

ble position. Doctor Jackson's Experimental Pharmacology should make friends among both instructors and students. It will take a load of detail from the shoulders of the busy instructor by means of the extended and careful descriptions of methods and apparatus.

A feature that is sure to appeal to the student is the very generous use of line drawings to illustrate even the simplest instruments and apparatus. For example, the tracheal cannula is illustated by three drawings, showing small, medium, and large-sized cannulas. Such simple and well-known laboratory equipment as the beaker, the evaporating dish, the battery jar, the scalpel, and the medicine dropper are illustrated. Four figures are given to show various sizes and styles of forceps. Each illustrated set-up, either simple or elaborate, is fully supplied with legends in large clear type. Parts of the more complicated apparatus in many cases are depicted in additional drawings. Colored plates are used to show innervation and blood vessels of various animals. Numerous reproductions of kymograph tracings show the student the kind of records that it is possible to secure. The text that goes with all of these illustrations is worthy of them. In simple, straightforward language the author tells what to do, something of what is to be looked for, and by frequent questions stimulates interest in the subject. The author writes with a frank, friendly style that is certain to win the confidence of the average student. See the following from A Note to the Student (p. 31):

> The student will often find it necessary to carry out his work with apparatus entirely different from that described in the text and often perhaps with an equipment which is exceedingly unsatisfactory. He should by no means be discouraged thereby, for much of the most valuable experimental work of all history has been performed with crude and unwieldy apparatus, and often under most discouraging circumstances. To accomplish much with little is a sure sign of ability and the medical student who approaches the subject of experimental pharmacology at the present time will find numerous opportunities to demonstrate his aptitude in this direction.

Part one of this book begins with a Preliminary Exercise in which the organization of the class into working groups and the assignment of tables and apparatus are outlined, followed by 168 experiments.

> The general anesthetics, being of fundamental importance for the progress of the course, are taken up first. Following this is a group of drugs chiefly characterized by their action on the central nervous system. After these come a series of substances possessing specific actions on some one or more parts of the involuntary nervous system. These are followed by drugs which act mainly on the circulatory system, then follow the antipy-

retics, a few miscellaneous drugs, and finally a few experiments on acids, alkalies, and some of the heavy metals.

The second part of the book contains two chapters, one on shop work and one on photography. These are chiefly of interest to the instructor, and it is advised that these be read in connection with the general preparation of apparatus, equipment, etc., for the course in pharmacology. [p. 25.]

A list of dealers in apparatus, tools, supplies, equipment, etc., and an index complete the volume.

The book is printed from large, clear type, and the experiment captions are distinguished by the use of heavy-faced type. The binding is such that the book remains open at any desired page without the necessity of breaking its back or using weights.

R. C. McG.

Commonwealth of Australia. | Quarantine Service. | Service Publication No. 3. | The History of Small-pox in Australia, 1788–1908 | Compiled from various sources by | J. H. L. Cumpston, M. D., D. P. H., Director of Quarantine for the | Commonwealth of Australia. | Issued under the authority of the | Hon. the Minister for Trade and Customs | 1914. | By authority: | Albert J. Mullett, Government Printer, Melbourne. | Paper, pp. 1–182.

Australia has indeed been fortunate in that all available data with regard to the visitations of smallpox over the entire continent and during more than a century can be presented with such a wealth of detail within the limits of one small volume and that the entire toll of life has been only a little over 500. Its good fortune is the more notable in that its relative freedom from the disease is not due to vaccination, the number of vaccinations officially recorded being only a little over 30 per cent of the births.

Vaccination acts were passed in South Australia in 1853, in Victoria and Tasmania in 1854, and in Western Australia in 1861, requiring the vaccination of infants within six or twelve months of birth. During most of the decade from 1890 to 1899 the Tasmania Act is stated to have been a dead letter, no funds having been provided for its enforcement in certain years, while in Western Australia it appears that the Act was never thoroughly enforced, and an amendment for the relief of conscientious objectors was added in 1911. South Australia had already taken similar action in 1901. It appears that compulsory vaccination has never been required in New South Wales and Queensland, though public vaccinators were appointed, and a Vaccine Institute was long maintained at Sydney. The main

source of lymph supply since 1883 has been the Vaccine Depot at Melbourne, Victoria.

The most serious single epidemic was that that occurred at Sydney from May, 1881, to February, 1882, with a record of 154 cases, 40 of which were fatal. The expenses to the state incident to this epidemic were in excess of 400,000 dollars. Thorough compulsory vaccination would have cut it short by several months, saved lives and suffering, and nine tenths of the expense.

A most interesting epidemiologic problem is presented, but not solved, in the work. Why, with probably more than half the population of Australia unprotected by vaccination, and a large proportion of the remainder only partially protected by a single vaccination in infancy, have the epidemics of smallpox been so easily controlled. Doctor Cumpston advances the hypothesis that "the controlling factor under Australian conditions has been the absence of sufficient aggregation of population to permit of the spread of the disease so rapidly as to become beyond control." Very good as far as it goes, but not very convincing when applied to populous capitals such as Melbourne and Sydney.

J. D. LONG.

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PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, NOVEMBER 5, 1917

The regular monthly meeting of the Manila Medical Society was held in the College of Medicine and Surgery on the evening of November 5, 1917, at 8.30, President Ruth presiding. There were 19 members and 2 visitors present.

The minutes of the last meeting were read and approved as read.

The application for active membership of Major J. H. H. Scudder, M. C., U. S. Army, which had been favorably considered by the council, was presented to the society for ratification. The society ratified the recommendation of the council, and the secretary was instructed to notify Major Scudder of his acceptance to active membership.

H. G. MAUL,
Secretary-Treasurer.

SCIENTIFIC PROGRAM

ABSCESS OF THE BRAIN IN A CHILD

By DR. MARIA MENDOZA-GUAZON

The brain of a male child, age 1.5 years, with an abscess in the central part of the left occipital lobe due to a staphylococcus infection, was shown. A well-developed pyogenic membrane was present.

SPECIMENS OF INTESTINAL LESIONS

By DR. B. C. CROWELL

Forty-six preserved intestinal specimens illustrating the lesions of typhoid fever and of amœbic and bacillary dysenteries were presented. These were suspended from a screen with "bulldog" paper clips for exhibition. Eighteen specimens showed the types of lesions encountered in typhoid fever, over half with ulceration of the colon (eight cases to a pronounced degree); the frequent location of lesions near the ileocæcal valve and the involvement of the appendix were noted. Seventeen specimens from cases of entamœbic dysentery were exhibited; two of these had superimposed lesions of bacillary dysentery. Duodenal ulcer was observed in two cases and gastric ulcer in

two other instances in this series. Eleven specimens were shown illustrating bacillary colitis, one case among these with superimposed cholera, a second with amœbic colitis, and a third with typhoid lesions.

INFECTIONS WITH COCCIDIUM AND ISOSPORA IN ANIMALS IN THE PHILIPPINE ISLANDS AND THEIR POSSIBLE CLINICAL SIGNIFICANCE

By Prof. Frank G. Haughwout

In the light of the recent discovery in Manila of several species of coccidia, the attention of the society was directed to the possibility of the infection of human beings by these parasites of the lower animals. Several instances were cited where protozoan parasites of lower animals had been found infesting man, and attention was called to recent human infections with *Coccidium* and *Isospora* in the war zone. The paper was illustrated by microscopical demonstrations showing stages in the life cycle of both *Coccidium* and *Isospora*, the pathological changes produced by them, and comparative material illustrating the ease with which eggs of helminths may be mistaken for the cysts of coccidia and vice versa.

PHILIPPINE CONTACT POISONS

By Prof. E. D. Merrill

There was a general discussion of contact poisons, not only those found in the Philippines, but those occurring in other countries. The talk was illustrated by the exhibition of herbarium specimens. The matter of those plants causing injuries by purely mechanical means was briefly mentioned, spines, bristles, etc.; the general characters of the nettle type of stinging hairs was discussed, with the structure of the hairs and their irritating contents. The fact was emphasized that all poisons of the contact type that cause violent skin eruptions, comparable to the *Rhus* or poison oak poisoning, were all from representatives of a single natural family, Anacardiaceæ, or mango family; including *Rhus*, *Semecarpus* (lacquer poisoning), *Mangifera* (mango), and *Gluta* (rengas). The poisonous principle in these plants is a nonvolatile, very permanent oil, highly irritating in character. Treatment indicated is washing the infected parts with alcohol or by treatment with lead acetate, the former acting as a solvent, the latter forming an insoluble compound with the toxicodendrol; vaseline and salves should never be used, as they merely spread the irritating oil. In conclusion, the stinging crystals or rhaphides of oxalate of lime,

as found in representatives of the Araceæ (*gabi* family), and certain palms were discussed; these cause intense irritation when brought in contact with the mucous membranes.

UNUSUAL LOCATION OF VACCINATION TAKE (DEMONSTRATION)

By Dr. O. Schöbl

An accidental vaccination on the hand resulted from a cut due to the breaking of an ampule containing vaccine, even though the wound was cleansed with 2 per cent lysol solution. Photographs taken at intervals of three days were presented to show the development and healing of the take, which was fully developed on the ninth day. A secondary take followed. The case is of particular interest because the patient had smallpox thirty-five years ago and because of repeated nonsuccessful vaccinations since that time.

R. B. Gibson,
Editor of the Proceedings.